8410506

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

September 7, 2023

THIS IS TO CERTIFY THAT ANNEXED IS A TRUE COPY FROM THE RECORDS OF THIS OFFICE OF THE FILE WRAPPER AND CONTENTS OF:

APPLICATION NUMBER: *16/541,071*
FILING DATE: *August 14, 2019*
PATENT NUMBER: *11,140,841*
ISSUE DATE: *October 12, 2021*

By Authority of the
Under Secretary of Commerce for Intellectual Property
and Director of the United States Patent and Trademark Office

Rodney Glover
Certifying Officer

PTO/AIA/15 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	VONL:004USC1
First Named Inventor	Willem Hendrik Lindhout
Title	Hybrid Seed Potato Breeding
Express Mail Label No.	Electronically filed via EFS Web

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. ☐ **Fee Transmittal Form** (PTO/SB/17 or equivalent)
2. ☑ **Applicant asserts small entity status.** See 37 CFR 1.27
3. ☐ **Applicant certifies micro entity status.** See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
4. ☑ **Specification** [*Total Pages* 75] Both the claims and abstract must start on a new page. *(See MPEP § 608.01(a) for information on the preferred arrangement)*
5. ☑ **Drawing(s)** (35 U.S.C. 113) [*Total Sheets* 11]
6. **Inventor's Oath or Declaration** [*Total Pages* ____] *(including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))*
   a. ☐ Newly executed (original or copy)
   b. ☐ A copy from a prior application (37 CFR 1.63(d))
7. ☑ **Application Data Sheet** ** See note below.* See 37 CFR 1.76 (PTO/AIA/14 or equivalent)
8. **CD-ROM or CD-R** in duplicate, large table, or Computer Program (*Appendix*)
   ☐ Landscape Table on CD
9. **Nucleotide and/or Amino Acid Sequence Submission** (*if applicable, items a. – c. are required*)
   a. ☐ Computer Readable Form (CRF)
   b. ☐ Specification Sequence Listing on:
      i. ☐ CD-ROM or CD-R (2 copies); or
      ii. ☐ Paper
   c. ☐ Statements verifying identity of above copies

ADDRESS TO:

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

ACCOMPANYING APPLICATION PAPERS

10. ☐ **Assignment Papers** (cover sheet & document(s))
    Name of Assignee ____
11. ☑ **37 CFR 3.73(c) Statement** (*when there is an assignee*) ☑ **Power of Attorney**
12. ☐ **English Translation Document** (*if applicable*)
13. ☑ **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    ☐ Copies of citations attached
14. ☑ **Preliminary Amendment**
15. ☐ **Return Receipt Postcard** (*MPEP § 503*) (*Should be specifically itemized*)
16. ☐ **Certified Copy of Priority Document(s)** (*if foreign priority is claimed*)
17. ☐ **Nonpublication Request** Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
18. ☑ **Other:** Request for Transfer of a Computer Readable Form Under 37 C.F.R. 1.821(e) and Certification and Request for Prioritized Examination Under 37CFR 1.102(e)

Note*: (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 **must be included in an Application Data Sheet (ADS).
(2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

19. CORRESPONDENCE ADDRESS

☑ The address associated with Customer Number: 125771 **OR** ☐ Correspondence address below

Name					
Address					
City		State		Zip Code	
Country		Telephone		Email	

Signature	/Ashley Hamlett/	Date	August 14, 2019
Name (Print/Type)	Ashley Hamlett	Registration No. (Attorney/Agent)	62,569

This collection of information is required by 37 CFR 1.53(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	VONL:004USC1
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Willem	Hendrik	Lindhout	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Wageningen	Country of Residence	NL

Mailing Address of Inventor:

Address 1	Delhorstpad 1A		
Address 2			
City	Wageningen	**State/Province**	
Postal Code	6703 BE	**Country**	NL

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Teunis	Philippus	Schotte	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Hazerswoude-Dorp	Country of Residence	NL

Mailing Address of Inventor:

Address 1	Westzijdeweg 33A		
Address 2			
City	Hazerswoude-Dorp	**State/Province**	
Postal Code	2391 JE	**Country**	NL

Inventor 3 Remove

Legal Name

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	VONL:004USC1
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

Prefix	Given Name	Middle Name	Family Name	Suffix
	Richard	Gerardus Franciscus	Visser	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Bennekom	Country of Residence	NL

Mailing Address of Inventor:

Address 1	Emmalaan 17		
Address 2			
City	Bennekom	State/Province	
Postal Code	6721 ET	Country	NL

Inventor 4 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Herman	Johannes	van Eck	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Wageningen	Country of Residence	NL

Mailing Address of Inventor:

Address 1	Eykmanstraat 9		
Address 2			
City	Wageningen	State/Province	
Postal Code	6706 JT	Country	NL

Inventor 5 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Ronaldus	Cornelis Bernardus	Hutten	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Rhenen	Country of Residence	NL

Mailing Address of Inventor:

Address 1	DeThijmenstoren 12		
Address 2			
City	Rhenen	State/Province	
Postal Code	3912 AT	Country	NL

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	VONL:004USC1
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	125771		
Email Address		Add Email	Remove Email

Application Information:

Title of the Invention	Hybrid Seed Potato Breeding		
Attorney Docket Number	VONL:004USC1	**Small Entity Status Claimed**	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	11	**Suggested Figure for Publication (if any)**	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	VONL:004USC1
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	125771		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Continuation of	13504156	2012-07-10
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
13504156	a 371 of international	PCT/NL10/50716	2010-10-26
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/NL10/50716	Claims benefit of provisional	61254980	2009-10-26

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

			Remove
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)
09013474.3	EP	2009-10-26	

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	VONL:004USC1
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	VONL:004USC1
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	VONL:004USC1
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◉ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
○ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	Agventure B.V.

Mailing Address Information For Applicant:

Address 1	Burg. Arriensweg 32		
Address 2			
City	Diepenveen	**State/Province**	
Country	NL	Postal Code	7431 BM
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button.

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	VONL:004USC1
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Ashley Hamlett/			Date (YYYY-MM-DD)	2019-08-14
First Name	Ashley	Last Name	Hamlett	Registration Number	62569

Additional Signature may be generated within this form by selecting the Add button.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	VONL:004USC1
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

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STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: Agventure B.V.

Application No./Patent No.: Filed herewith Filed/Issue Date: Filed herewith

Titled: Hybrid Seed Potato Breeding

Agventure B.V., a CORPORATION

(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. ☑ The assignee of the entire right, title, and interest.

2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):

   ☐ The extent (by percentage) of its ownership interest is _____%. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

   ☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☐ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. ☐ The recipient, via a court proceeding or the like (*e.g.*, bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

A. ☑ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 028529, Frame 0059, or for which a copy thereof is attached.

B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

   1. From: _____ To: _____

      The document was recorded in the United States Patent and Trademark Office at

      Reel _____, Frame _____, or for which a copy thereof is attached.

   2. From: _____ To: _____

      The document was recorded in the United States Patent and Trademark Office at

      Reel _____, Frame _____, or for which a copy thereof is attached.

This collection of information is required by 37 CFR 3.73(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

4. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

5. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

6. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☑ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Ashley Hamlett/	August 14, 2019
Signature	Date
Ashley Hamlett	62,569
Printed or Typed Name	Title or Registration Number

PTO/AIA/82 (07-13)
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POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(c).

I hereby appoint:

[x] Practitioners associated with Customer Number: 125771

OR

[ ] Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used)

Name	Registration Number	Name	Registration Number

As attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignment documents attached to this form in accordance with 37 CFR 3.73(c).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(c) to:

[x] The address associated with Customer Number: 125771

OR

[ ] Firm or Individual Name

Address

City | State | Zip

Country

Telephone | Email

Assignee name and address: Aardevo B.V.
Rijnzathaven 16
Zwijndrecht, Netherlands NL- 3371 BW

A copy of this form, together with a statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is required to be filed in each application in which this form is used. The statement under 37 CFR 3.73(c) may be completed by one of the practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

Signature | Date: 7/24/2014

Name: [illegible] | Telephone: +31 (0)78 6116 211

Title: CEO

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to be filed by the USPTO to prosecute the application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 18 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**
If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	Hybrid Seed Potato Breeding
First Named Inventor/Applicant Name:	Willem Hendrik Lindhout
Filer:	Ashley Greene Hamlett
Attorney Docket Number:	VONL:004USC1

Filed as Small Entity

Filing Fees for Track I Prioritized Examination - Nonprovisional Application under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
UTILITY FILING FEE (ELECTRONIC FILING)	4011	1	75	75
UTILITY SEARCH FEE	2111	1	330	330
UTILITY EXAMINATION FEE	2311	1	380	380
REQUEST FOR PRIORITIZED EXAMINATION	2817	1	2000	2000
Pages:				
Claims:				
Miscellaneous-Filing:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
PUBL. FEE- EARLY, VOLUNTARY, OR NORMAL	1504	1	0	0
PROCESSING FEE, EXCEPT PROV. APPLS.	2830	1	70	70

Petition:	
Patent-Appeals-and-Interference:	
Post-Allowance-and-Post-Issuance:	
Extension-of-Time:	
Miscellaneous:	
Total in USD ($)	**2855**

Electronic Acknowledgement Receipt

EFS ID:	36878687
Application Number:	16541071
International Application Number:	
Confirmation Number:	4348
Title of Invention:	Hybrid Seed Potato Breeding
First Named Inventor/Applicant Name:	Willem Hendrik Lindhout
Customer Number:	125771
Filer:	Ashley Greene Hamlett
Filer Authorized By:	
Attorney Docket Number:	VONL:004USC1
Receipt Date:	14-AUG-2019
Filing Date:	
Time Stamp:	20:03:58
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$2855
RAM confirmation Number	E20198DK06060501
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	TrackOne Request	VONL_004USC1_Track_1_Req.pdf	102572 3da0b099d49151d4c6f42058d991e3e25d4388d9	no	1
Warnings:					
Information:					
2		VONL_004USC1_Specification.PDF	5153918 6b24607a9f367c6bddeaae3437c2f70f7165ffdc	yes	86

Multipart Description/PDF files in .zip description

Document Description	Start	End
Specification	1	67
Claims	68	73
Drawings-only black and white line drawings	74	84
Abstract	85	86

Warnings:					
Information:					
3		VONL_004USC1_Prelim_Amend.pdf	35961 2e32941b99752ee537d8c1930c079c3569f72476	yes	6

Multipart Description/PDF files in .zip description

Document Description	Start	End
Preliminary Amendment	1	1
Specification	2	2
Claims	3	5

	Applicant Arguments/Remarks Made in an Amendment		6	6	
Warnings:					
Information:					
4	Request for Transfer of a Computer Readable Form	VONL_004USC1_Req_for_Trans.pdf	186456 d25cf4c07dfe12bc85add2d7c161bc8bad7534e5	no	33
Warnings:					
Information:					
5		VONL_004USC1_IDS.pdf	161455 5c06a3922a756ab3bbe4797265eb8a37072e3c4e	yes	12
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Transmittal Letter		1	2	
	Information Disclosure Statement (IDS) Form (SB08)		3	12	
Warnings:					
Information:					
6	Transmittal of New Application	VONL_004USC1_Transpto.pdf	139410 6b46bd587fa83fee903af562c012a3ae888d2c9a	no	1
Warnings:					
Information:					
7	Application Data Sheet	VONL_004USC1_ADSpto.pdf	118665 091a87a2e915c52e709f335323d0c192238514aa	no	9
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
8		VONL_004USC1_3_73_Stmtpto.pdf	838899 8e9f9e216216ee43518e32527554b9ad41c8e839	yes	3
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	

	Miscellaneous Incoming Letter		1		2
	Power of Attorney		3		3
Warnings:					
Information:					
9	Fee Worksheet (SB06)	fee-info.pdf	40217 4d765b6ee6d4727a3d6d9344c2c0bba514895d48	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	6777553		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Doc Code: TRACK1.REQ
Document Description: TrackOne Request

PTO/AIA/424 (04-14)

CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

First Named Inventor:	Willem Hendrik Lindhout	Nonprovisional Application Number (if known):	Filed Herewith
Title of Invention:	Hybrid Seed Potato Breeding		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1) and the prioritized examination fee set forth in 37 CFR 1.17(c) have been filed with the request. The publication fee requirement is met because that fee, set forth in 37 CFR 1.18(d), is currently $0. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.

2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims, and that any request for an extension of time will cause an outstanding Track I request to be dismissed.

3. The applicable box is checked below:

I. ☑ Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.

---OR---

(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, **or** the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. ☐ Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

i. A request for continued examination has been filed with, or prior to, this form.
ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature /Ashley Hamlett/	Date August 14, 2019
Name (Print/Typed) Ashley Hamlett	Practitioner Registration Number 62,569

Note: *This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.**

☐ *Total of _____ forms are submitted.

Title: Hybrid Seed Potato Breeding

FIELD OF THE INVENTION

The invention relates to the field of agriculture, in particular to new plants and processes for obtaining them. The invention also relates to methods for improving the genetic constitution of crop plants, and to plants thus obtained. The invention further relates to a method for producing hybrid seeds and to hybrid seeds thus produced and to methods of producing crops using the hybrid seeds of the invention.

BACKGROUND OF THE INVENTION

The potato (*Solanum tuberosum* L.) is an integral part of the global food system. It is the world's number one non-grain food commodity, with production reaching a record 325 million tonnes in 2007.

Unlike other major field crops, potatoes are reproduced vegetatively from other potatoes. Therefore, a part of each year's crop - ranging from 5 to 15 percent, depending on the quality of the harvested tubers - is set aside for re-use in the next planting season. Most farmers in developing countries select and store their own seed tubers. In developed countries, farmers are more likely to purchase disease-free "certified seed" from dedicated suppliers.

Seed potatoes are more difficult to produce and supply than grain or pulse seed. A seed:harvest ratio of 1:20 for potatoes is considered good, compared to 1:400 for maize or 1:10.000 for tomato. One hectare may therefore require two tonnes of seed material to maximize the yield of harvestable products, compared to 18 kg for maize. In order to break dormancy, seed potatoes should be stored for several weeks before they can be planted. The right conditions during storage such as amount of light, temperature and humidity are crucial to ensure good "seed" quality.

In addition to the poor seed:harvest ratio, seed potatoes attract and transport pests and diseases. These include (amongst others) late blight, Andean potato weevils, nematodes, tuber moths and viruses. The latter are transmitted in the field by aphids and then carried from generation to generation in the seed. Such a virus infection can decrease yields by up to 20 percent.

Seed potatoes have high transportation costs because of the great distances between the major seed production areas and the major consumer production areas and the relatively high weight of individual seed potato tubers.

To feed the growing world population now and in future, the potato industry has to keep growing to meet the needs of the consuming public. Substantial research and development efforts are devoted to the modernization of planting and harvesting of fields and processing of potatoes, and to the development of economically advantageous potato varieties. Through crossbreeding of potatoes, researchers hope to obtain potatoes with the desirable characteristics of good processing, both for fresh consumption as well as for industrial purposes, high soluble solids content, high yield, resistance to diseases and pests and adaptability to various growing areas and conditions.

The research leading to potato varieties which combine the advantageous characteristics referred to above is largely empirical. This research requires large investments of time, manpower, and money. The development of a potato cultivar can often take up to eight years or more followed by at least five years of propagation to get sufficient quantities for commercial usage. Breeding begins with careful selection of superior parents to incorporate the most important characteristics into the progeny. Since all desired traits usually do not appear in one progeny, breeding is a continuous process of selecting the best recombinants, combining favourable traits of the ancestors.

The arduous task of producing a new potato variety is best understood when understanding the genetics of potato. The commercial potato has a tetraploid genome. Diploid tubers are generally too small for important commercial applications. In addition, the tetraploid genome is extremely heterozygous, often harbouring multiple alleles per locus. It is believed that self-incompatibility, which is primarily experienced at diploid level, and inbreeding depression are responsible for the maintenance of the high genetic variability found in potato and that overdominance of heterozygous alleles (heterosis) results in vigorous plants. In a typical potato offspring obtained from a cross between two unrelated parental lines deleterious alleles may therefore contribute to either a reduced fitness in case of homozygosity or an increased vigour in case of heterozygosity. It is clear that a breeder needs large populations to maximise the chance of finding individuals that carry a relatively high

number of heterozygotic loci and a low number of homozygotic loci, while also exhibiting beneficial combinations of agronomically desirable traits.

Present potato breeding techniques rely on the controlled crossing of parental clones which themselves are the result of a comprehensive pre-breeding development during which amongst others special techniques such as chromosome doubling, embryo rescue, and somatic fusion are applied in order to introduce the beneficial characteristics of for instance wild and primitive *Solanum* species into these clones. The parental material that is found suitable for further breeding after a phenotypic selection procedure is then mutually crossed and the resulting non-uniform hybrid seeds are sown in large numbers in greenhouses. From tens of thousands of individual F1 seedlings tubers are harvested and retained for the next year's planting. The next year a single "seed" tuber from each resulting seedling is planted in the field. Extreme caution must be taken to avoid the introduction of viruses and diseases since the material is only clonally (vegetatively) expanded before it is sold to individual customers years later. After the second year, samples of tubers are taken for density measurements and preliminary fry tests to determine the suitability of the tubers for commercial use. A multitude of tubers of plants which have survived the selection process to this point are then planted in the third year for a more comprehensive series of fry tests and density determinations. At the fourth-year stage of development, a diminishing number of surviving selections is grown in ever expanding numbers and plants thereof are subjected to field trials in several stages to determine their adaptability to different growing conditions. Eventually, the varieties having superior agronomical qualities are transferred to other farms and the "seed" (in the form of tubers) is increased to commercial scale. Since one "seed" tuber may generate between 6 and 20 harvested tubers this up-scaling process may take years before sufficient "seed" is produced. Generally, by this time, eight or more years of planting, harvesting and testing have been invested in attempting to develop a new and improved potato cultivar.

To reduce inbreeding depression a breeder may introduce new genes from a genetically more remote parent such as from wild and primitive species with ploidy levels ranging from diploid to hexaploid. However, when two genetically unrelated potato plants are crossed, the level of heterozygosity may be increased but simultaneously more deleterious genes are also introduced. As a consequence, a

breeder will typically make additional crosses with more commercial germplasm to enrich the population for favourable alleles. All together such a multiple crossing breeding programme may take dozens of years as the selection of the favourable genotypes in each generation may already take five years. Therefore, potato breeding is currently a predominantly empirical exercise, strongly characterised by trial and error.

Potatoes and their related wild species (tuber-bearing *Solanum* species) are mostly outbreeding because self fertilisation is hampered by a gametophytic self-incompatibility system. Self-incompatibility (SI) is a general name for several genetic mechanisms in angiosperms, which prevent self-fertilization and inbreeding. In plants with SI, when a pollen grain produced in a plant reaches a stigma of the same plant or another plant with a similar genotype, the process of pollen germination, pollen tube growth, ovule fertilization, and embryo development is halted at one of its stages, and consequently no seeds are produced. Self-incompatibility is not found in tetraploid potatoes.

The provision of such self-compatible clones may facilitate the generation of selfed progenies of potato, and hence the production of (highly) homozygous potato lines. This could provide a great opportunity to the development of homozygous elite breeding lines in potato. However, to date, the development of homozygous elite lines with genetically fixed agronomically desirable traits, that would enable the production of genetically uniform hybrid potato seed, has been unsuccessful.

The provision of homozygous elite lines is hampered by unknown causes. Selfing of an occasionally encountered self-compatible clone results in a slower decrease of heterozygosity than theoretically expected. The slower decline of heterozygosity may be the result of unintentional but unavoidable selection during selfing for the heterozygous plants in the offspring that exhibit higher vigour, fertility and seed germination. It is implied that, as the heterozygosity is reduced by selfing, the fertility and vigour are also reduced and the plants may become weak and completely sterile. The situation is perhaps worsened by the establishment of homozygous configurations of recessive deleterious genes. This phenomenon which is generally referred to as inbreeding depression has greatly hampered the development for homozygous potato lines and hence the production of uniform hybrid potato seed.

There is a large prejudice against the production of homozygous breeding lines in potato due to inbreeding depression. Uijtewaal et al. (Euphytica 36 (1987) 745-753) indicated that owing to sterility problems, homozygous potato clones would be of little importance for practical breeding. Developing homozygous inbred lines was considered, but rendered impossible in potato (Umaerus, 1987, Proceeding of the 10th Triennial Conference of the European Association of Potato Research, Aalborg, Denmark, pp 72–103 as cited in Almekinders et al. 2009 Potato Research 52:275–293). Routes involving doubling of haploids have long been presumed as promising. Nontheless, up to the present day the ruling opinion is that inbreeding depression in diploid potato is too strong to ever result in vigorous homozygous plants.

Birhman and Hosaka (Genome 43: 495–502 (2000)) have suggested the possibility to utilize the *Sli* gene derived from *S. chacoense* for development of highly homozygous true potato seed (TPS) lines and heterosis breeding of the potato. However, to date, no homozygous lines with good agronomic traits such as good tuber yield, have ever been reported from this proposed line of research. Rather, those homozygotes that have been produced do not exhibit any agronomically relevant tuber yield.

Rommens in 2010 (Genetic modification of Plants, Kempen & Jung , eds, In: Biotechnology in Agriculture and Forestry 64(1): 61-77 (2010)), advocates the route of genetic transformation, due to the fact that efforts to improve the yield and quality of this crop are hampered by inbreeding depression.

In short, production of true breeding lines of potato is considered impossible. Due to this fact, potato breeding cannot escape the traditional schemes based on crossing of tetraploid heterozygotes. As a result, the challenge is considered formidable to combine various utilization traits (relating to fresh and processing uses), resistances to pathogens and pests, and numerous other relevant agronomic traits with improvements in yield into a commercially acceptable cultivar (Douches et al. 1999, Crop Science 36(6):1544-1552).

It is an objective of the present invention to provide means and methods for the production of elite breeding lines of potato and for the production of uniform hybrid potato seed from which plants can be grown that exhibit agronomically relevant tuber yield.

SUMMARY OF THE INVENTION

The present inventors have attempted to achieve homozygous parental breeding lines for generative propagation of potatoes via a number of routes, none of which were successful. These unsuccessful routes included androgenesis, gynogenesis, doubling of haploids and continued selfings. Only by repeated selfing of a self-compatible plant comprising the *Sli* gene derived from *S. chacoense* and the use of did the inventors arrive at the product of the present invention. The present invention now provides in a first aspect a diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant. Plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant are in general terms also referred to herein as being vigorous.

In a preferred embodiment, said line in addition to said tuber yield comprises at least one, more preferably at least 2, 3, 4, 5 or 6 (additional) agronomically desirable traits. Preferably, said agronomically desirable traits are selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, and cold sweetening resistance.

In another preferred embodiment of said potato line of the invention, plants of said line when mature attain an average fresh weight of the foliage and shoots of at least 500 grams per plant. Plants attaining an average fresh weight of the foliage and shoots of at least 500 grams per plant are in general terms also referred to herein as being vigorous.

In another aspect, the present invention provides a plant of a line according to the invention as described above. Preferably, said plant is diploid, fertile, self-compatible and essentially homozygous and has an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant.

In another aspect, the present invention provides a seed of a plant as described above. Said seed may be homozygous (i.e. represent a selfed seed), but may

also be heterozygous for the alleles where the parents differ. In one embodiment, said seed is preferably a uniform hybrid potato seed. Such uniform hybrid potato seed being the result of a cross between two homozygous, self-compatible, fertile and vigorous plants of a line according to the invention as described above, preferably of two different lines, wherein the lines contain at least 20 % contrasting homozygous loci as determinable by molecular marker analysis.

In another aspect, the present invention provides a method for producing a diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, comprising:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, self-compatible, fertile and essentially homozygous potato line;

(b) providing a second potato plant, wherein said second potato plant is a plant of a diploid or tetraploid potato line which may have any level of homozygosity, said line of said second potato plant comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant;

(c) cross pollinating said first and second potato plant to provide seeds and collecting said seeds to thereby provide an offspring generation in the form of a hybrid offspring seed;

(d) growing said hybrid offspring seed into a population of hybrid offspring potato plant and selecting from said population plants producing tubers at a yield expressed in grams of fresh weight of at least 200 grams per plant, and

(e) selfing said hybrid offspring potato plant or backcrossing said hybrid offspring potato plant to a potato plant of said line of said first potato plant, for between 1 to 8 selfings or backcrosses, to thereby provide a diploid, self-compatible and essentially homozygous potato breeding line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant.

A first potato plant in a method as described herein is preferably a plant of a first diploid, self-compatible, fertile and essentially homozygous potato line, wherein the self-compatibility is conditioned by the presence of a dominant allele of an S-locus (self-incompatibility locus) inhibitor gene (Sli) that is nonallelic to the S locus as described in Hosaka and Hanneman 1998 Euphytica, 99: 191–197.

The above method, when performed on a sufficiently large scale (at least more than 10.000 plants) and performed with the use of a suitable second potato plant, which is preferably a diploid potato clone that is essentially not homozygous, said line of said second potato plant comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, preferably a line such as IVPAA-096-18 (D2) as described in the Examples herein below, results in the production of an essentially homozygous, fertile, vigorous and self-compatible breeding line, wherein said vigour is in particular represented by an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant. The provision of such a breeding line is a crucial step in the provision of genetically homozygous true potato seed (TPS).

The first and second breeding line can subsequently be used in the production of the TPS when both the first and second breeding line comprise agronomically desirable characteristics, have good general combining abilities, and preferably have at least 20% contrasting homozygous loci as determinable by molecular marker analysis.

In a preferred embodiment of said method for producing a diploid, fertile, self-compatible and essentially homozygous potato line according to the invention, said steps a-e are repeated wherein step b comprises the provision of third (or further) potato plant wherein said third potato plant is a plant of a diploid or tetraploid potato line which may have any level of homozygosity, wherein said third potato plant is a donor for an additional agronomically desirable trait. Preferably, said trait is selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, and cold sweetening resistance, or any other agronomically beneficial trait or desirable characteristic as defined herein.

The second or third potato plant as described above may suitably be a diploid potato clone selected from the plants D1-D21 as listed in Table 6 of Example 3 below. The skilled person will understand that other diploid potato clones may also be used. Highly preferred is the use of potato clone IVPAA-096-18 (D2) which is available

from Laboratory of Plant Breeding (IVP) (Dr. Ir Ronald B.C. Hutten), Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.

Proper (commercial) sources of tetraploid cultivars are provided through the European Cultivated Potato Database (ECPD) at http://www.europotato.org/menu.php and/or the Potato Pedigree Database of the expertise group Plant Breeding (a cooperation between the Laboratory of Plant breeding of Wageningen UR, the Netherlands) and the business unit Biodiversity and breeding of Plant Research International (PRI), Wageningen, the Netherlands at http://www.plantbreeding.wur.nl/potatopedigree/.

In another preferred embodiment of said method, said first potato plant is a plant of potato line NCIMB 41663, NCIMB 41664, NCIMB 41665, or NCIMB 41765, representative seeds of said line having been deposited with the NCIMB, Aberdeen, Scotland.

In yet another preferred embodiment of said method, said second potato plant is a plant of a second diploid, fertile, self-compatible and essentially homozygous potato line.

In yet a further preferred embodiment of said method, said second potato plant is a plant of a second diploid, fertile, self-incompatible potato line.

The method for producing a diploid potato breeding line may further comprise the steps of enhancing the breeding value of the diploid potato breeding line by intercrossing, selfing and backcrossing while continuous selecting for agronomically desirable traits. Experimental crosses are done to evaluate general combining abilities (intercrossability).

In another aspect, the present invention provides a diploid potato breeding line, more preferably an elite line, obtainable by the method of the present invention as described above.

In another aspect, the present invention provides a hybrid potato seed obtainable by crossing plants of a first and second diploid potato breeding line according to the present invention as described above, wherein said plants of said first and second diploid potato breeding line contain at least 20 % contrasting homozygous loci as determinable by molecular marker analysis.

In another aspect, the present invention provides a method for producing a uniform hybrid potato seed, comprising:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, fertile, self-compatible and essentially homozygous potato line,

(b) providing a second potato plant, wherein said second potato plant is a plant of a second diploid, fertile, self-compatible and essentially homozygous potato line, wherein said first and second plant contain at least 20 % contrasting homozygous loci as determinable by molecular marker analysis, and

(c) cross pollinating said first and second potato plant and allowing one of said parent plants to produce berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed.

In a preferred embodiment of a method for producing hybrid seed, said first and second potato plant are from potato lines comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, preferably wherein said first and second potato plant are plants having a tuber yield of at least 200 grams fresh weight per plant.

In another preferred embodiment of said method of producing hybrid seed, at least one of said first and second potato plants comprises at least one agronomically desirable trait, preferably selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, and cold sweetening resistance.

In another preferred embodiment of a method for producing hybrid seed according to the present invention, said first potato line is a diploid, self-compatible and essentially homozygous offspring potato plant comprising the *Sli*-gene, preferably wherein said first potato plant is a plant of potato lines NCIMB 41663, NCIMB 41664, NCIMB 41665, or NCIMB 41765, representative seeds of said lines having been deposited with the NCIMB, Aberdeen, Scotland, or a diploid, self-compatible and essentially homozygous offspring potato plant thereof.

In another preferred embodiment of a method for producing hybrid seed according to the present invention, said second potato plant is a plant of a second diploid, self-compatible and essentially homozygous potato line preferably comprising at least two agronomically desirable traits selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, and cold sweetening resistance.

In another aspect, the present invention provides a seed of potato lines NCIMB 41663, NCIMB 41664, NCIMB 41665, or NCIMB 41765, representative seeds of said line having been deposited with the NCIMB, Aberdeen, Scotland.

In another aspect, the present invention provides a seed produced by the method for producing a uniform hybrid potato seed as disclosed herein.

In another aspect, the present invention provides a hybrid potato plant, or part thereof, produced by growing the seed of the present invention.

In another aspect, the present invention provides pollen or an ovule of the hybrid potato plant, or part thereof, of the present invention.

In another aspect, the present invention provides a tuber from the hybrid potato plant of the present invention.

In another aspect, the present invention provides a potato plant having all of the physiological and morphological characteristics of the hybrid potato plant produced by growing the seed of the present invention.

In another aspect, the present invention provides a tissue culture of the hybrid potato plants of the present invention.

In another aspect, the present invention provides a potato plant regenerated from the tissue culture of the present invention, wherein said regenerated potato plant has all the physiological and morphological characteristics of the hybrid potato plant produced by growing the seed of the present invention.

In another aspect, the present invention provides a method for producing a hybrid potato seed wherein the method comprises crossing a first potato plant with a second potato plant and harvesting the resultant hybrid potato seed, wherein said first and/or second parent potato plant is a diploid, fertile, self-compatible and essentially homozygous plant according to the present invention that has an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant.

In another aspect, the present invention provides a method of producing a hybrid potato plant, said method comprising producing a seed according to a method of the present invention and growing said seed into a potato plant.

In another aspect, the present invention provides a potato plant produced by the method of the invention, or a tuber thereof.

In another aspect, the present invention provides a method set of at least 4 intercrossable homozygous diploid, fertile, self-compatible and essentially homozygous potato lines comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant according to the present invention, wherein each of said lines differ from another line in said set by having at least 20 % contrasting homozygous loci as determinable by molecular genetic marker analysis.

The invention also provides as an embodiment of methods of the invention as described above methods comprising the steps of:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, self compatible and essentially homozygous potato line carrying chromosomes with alleles for agronomically desirable traits that are to be expressed in an offspring generations,

(b) providing a second potato plant, wherein said second potato plant is a plant of a second diploid, self compatible and essentially homozygous potato line carrying chromosomes with alleles for agronomically desirable traits that are to be expressed in an offspring generations and that may be different form the traits in the said first plant, and

(c) cross pollinating said first and second potato plant such that one of said parent plants produces berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed carrying combinations of chromosomes with said alleles.

Thus, in an alternative embodiment the plant of the first potato line may carry chromosomes with alleles for agronomically desirable traits that are to be expressed in an offspring generations, in which case the agronomically desirable trait in the plant of the second potato line may be different from the traits in the said first plant.

In another aspect, the present invention provides a method of producing a herbicide resistant, nematode resistant, insect resistant and/or disease resistant potato plant, said method comprising producing a seed according to a method of the invention described above by crossing parental lines that are herbicide resistant, nematode resistant, insect resistant and/or disease resistant and growing said seed a potato plant.

In another aspect, the present invention provides a herbicide resistant, nematode resistant, insect resistant and/or disease resistant potato plant produced by the method of the invention as described above.

In yet another aspect, the present invention provides a method for preventing transmittal of virus infections between potato generations, comprising:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, self-compatible and essentially homozygous potato line,

(b) providing a second potato plant, wherein said second potato plant is a plant of a potato line that may have any level of ploidy and/or any level of homozygosity, said second potato plant carrying a chromosome with an allele for a agronomically desirable trait that is to be expressed in an offspring generation;

(c) cross pollinating said first and second potato plant such that one of said parent plants produces berries with seeds and collecting said seeds from said berries

to provide an offspring generation in the form of a hybrid offspring seed carrying a chromosome with said allele;

(d) growing said hybrid offspring seed into a hybrid offspring potato plant carrying a chromosome with said allele, and

(e1) harvesting tubers from said hybrid offspring potato plant to provide tubers expressing said trait, or

(e2) selfing said hybrid offspring potato plant or backcrossing said hybrid offspring potato plant to a potato plant of said line of said first potato plant, thereby providing further offspring plants carrying a chromosome with said allele and wherein said offspring plants are increasingly homozygous following each backcrossing or selfing step.

In another aspect, the present invention provides a diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, said plant being obtainable by:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, fertile, self-compatible and essentially homozygous potato line,

(b) providing a second potato plant, wherein said second potato plant is a plant of a diploid potato line having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant,

(c) cross pollinating said first and second potato plant and allowing one of said parent plants to produce berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed,

(d) growing said hybrid offspring seed into a hybrid offspring potato plant,

(e) backcrossing said hybrid offspring potato plant to a potato plant of said line of said first potato plant, and selecting from the offspring plants thus generated a plant that is diploid, self-compatible and vigorous in that it produces tubers at a yield expressed in grams of fresh weight of at least 200 grams per plant, and

(f) selfing said diploid, self-compatible and vigorous plant for between 1 to 8 generations while selecting for self-compatibility and vigour as defined in (e).

In another aspect, the present invention provides a hybrid potato seed which seed, when sown produces a diploid, fertile, self-compatible and essentially heterozygous potato plant having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, said plant being obtainable by:

(a) providing a first diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant,

(b) providing a second diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, wherein said first and second plant contain at least 20 % contrasting homozygous loci as determinable by molecular marker analysis, and

(c) cross pollinating said first and second potato plant and allowing at least one of said parent plants to produce berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed.

The steps of selecting or providing plants that are homozygous, or the step of selecting or providing plants having at least 20% contrasting loci as defined herein above, may comprise marker assisted selection using the molecular genetic markers as provided herein.

DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic presentation of a development scheme for the development of hybrid potato cultivars using the methods and plants of the present invention, wherein the various steps in obtaining commercial potato hybrids are indicated:

0, Potato germplasm: The origin comprises in principle all potato genotypes within the cultivated potato species (*Solanum tuberosum*) and related crossable species;

1, Self-compatible diploid potato: Cultivated potato (*Solanum tuberosum*) is usually self incompatible. Self-compatibility is introduced from related species (see for instance Hermsen, J.G.Th. 1978, Euphytica 27, 1-11);

2, Homozygous fertile self-compatible diploid potato: Homozygous diploids can be obtained by doubling haploids (Uijtewaal et al., 1987, Theor. Appl. Genet. 73,

751-78) by anther culture (Jacobsen and Sopory, 1977, Theor. Appl. Genet. 52, 119-123), by ovule culture or by repeated selfings (Phumichai & Hosaka, 2006, Euphytica 149, 251-258). The example of the latter is illustrated herein;

3, Vigorous fertile homozygous self-compatible diploid potato: The vigour is enhanced by crossing with other diploid potato, selfing and backcrossing while selecting for higher vigour;

4, Diploid potato breeding Lines: Agronomically desirable traits are introduced by crossing with other diploid potato, selfing and backcrossing while selecting for these traits;

5, Elite diploid potato breeding Lines: The breeding value is further enhanced by intercrossing, selfing and backcrossing while continuous selecting for agronomically desirable traits. Experimental crosses are done to evaluate general combining abilities;

6 Potato hybrid cultivars: Good combining breeding lines are crossed. The seeds obtained are hybrid true potato seeds that are uniform.

Figure 2 shows a schematic presentation of the pedigrees of the populations, deposits and plants as described in the Examples, starting from a cross between a diploid, essentially homozygous and self-compatible potato plant ("H") and three different diploid clones (D1, D2, and D3) that are essentially non-homozygous and self-incompatible and that act as donor lines of one or more desirable agronomic traits. The production of an array of essentially homozygous lines from backcross lines to fully selfed clones, each having one or more desirable traits derived from the donor lines provides a set of elite breeding lines for the production of an array of hybrid potato cultivars in the form of hybrid seed. ⊗ Refers to selfings. As can be seen, and as detailed in the Examples, only donor line D2 resulted in the production of a large number of self-compatible plants that would show vigour as defined herein. Vigorous plants could be obtained from the other crossings as well, albeit at a much lower frequency (0.1-1%).

Figure 3 displays the ultra-dense genetic linkage map of the genome of diploid breeding clone RH89-039-16. The number on the left of the linkage group indicates the cumulative number of recombination events counted from the top. The number of AFLP markers in each bin is represented by shades of grey according to the figure legend. Putative centromere positions are indicated with "Cen" alongside the

chromosome (van Os et al., 2006 Genetics 173(2):1075-1087). The numbers on the right of the linkage group indicate the approximate location of the corresponding PotSNPmarker as used herein, wherein reference is made to Table 5B in Example 3, below, for marker annotations. The figure clearly indicates the distribution of informative markers over the potato genome of this clone. A similar distribution was observed for these markers with another clone used herein, clone SH83-92-488.

Figure 4 shows marker data of 100 markers over 20 different diploid potato genotypes, representing the diploid potato breeding germplasm (Example 2).

Figure 5 shows the result of SNP marker analysis as described in Example 3 on a set of six F3 populations and their respective parents.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

The term "potato" is used herein to refer to material that is essentially of species *Solanum tuberosum*, but may include introgression segments of other tuber bearing *Solanum* species such as *Solanum chacoense, Solanum phureja, Solanum andigena, Solanum demissum*.

The term "potato plant" is used herein to refer to a seedling or mature plant as grown from cell culture or seed. Persons of ordinary skill in the art will recognize that when the term "potato plant" is used in the context of the present invention, this also includes derivative varieties that retain the essential distinguishing characteristics of for instance potato varieties that are subject of the present invention, such as a Single Gene Converted plant of that variety or a transgenic derivative having one or more value-added genes incorporated therein (such as herbicide or pest resistance). Backcrossing methods can be used with the present invention to improve or introduce a characteristic into the variety.

The term "crossing" as used herein refers to the fertilization of female plants (or gametes) by male plants (or gametes). The term "gamete" refers to the haploid reproductive cell (egg or sperm) produced in plants by mitosis from a gametophyte and involved in sexual reproduction, during which two gametes of opposite sex fuse to form a diploid zygote. The term generally includes reference to a pollen (including the sperm cell) and an ovule (including the ovum). "Crossing" therefore generally refers to the fertilization of ovules of one individual with pollen

from another individual, whereas "selfing" refers to the fertilization of ovules of an individual with pollen from the same individual. Crossing is widely used in plant breeding and results in a mix of genomic information between the two plants crossed one chromosome from the mother and one chromosome from the father. This will result in a new combination of genetically inherited traits. Usually, the progeny of a crossing is designated as: "F1". If the F1 is not uniform (segregates) it is usually designated as "F1 population". "Selfing" of a homozygous plant will usually result in a genetic identical plant since there is no genetic variation. "Selfing" of an F1 will result in an offspring that segregates for all traits that have heterozygotic loci in the F1. Such offspring is designated: "F2" or "F2 population"

When referring to "crossing" in the context of achieving the introgression of a genomic region or segment, the skilled person will understand that in order to achieve the introgression of only a part of a chromosome of one plant into the chromosome of another plant, it is required that random portions of the genomes of both parental lines will be recombined during the cross due to the occurrence of crossing-over events in the production of the gametes in the parent lines. Therefore, the genomes of both parents must be combined in a single cell by a cross, where after the production of gametes from said cell and their fusion in fertilization will result in an introgression event.

The term "intercrossable", as used herein, refers to the ability to yield progeny plants after making crosses between parent plants.

As used herein, the terms "introgressing", "introgress" and "introgressed" refer to both a natural and artificial process whereby individual genes or entire chromosomes are moved from one individual, species, variety or cultivar into the genome of another individual, species, variety or cultivar, by crossing those individuals, species, varieties or cultivars. In plant breeding, the process usually involves selfing or backcrossing to the recurrent parent to provide for an increasingly homozygous plant having essentially the characteristics of the recurrent parent in addition to the introgressed gene or trait.

The term "introgression" refers to the result of an introgression event.

The term "backcross" refers to the result of a "backcrossing" process wherein the plant resulting from a cross between two parental lines is (repeatedly) crossed with one of its parental lines, wherein the parental line used in the backcross

is referred to as the recurrent parent. Repeated backcrossing results in replacement of genome fragments of the donor parent with those of the recurrent. The offspring of a backcross is designated "BCx" or "BCx population", where "x" stands for the number of backcrosses.

The term "backcrossing" as used herein refers to the repeated crossing of a hybrid progeny back to the recurrent parents. The parental potato plant which contributes the gene for the desired characteristic is termed the nonrecurrent or donor parent. This terminology refers to the fact that the nonrecurrent parent is used one time in the backcross protocol and therefore does not recur. The parental potato plant to which the gene or genes from the nonrecurrent parent are transferred is known as the recurrent parent as it is used for several rounds in the backcrossing protocol. In a typical backcross protocol, the original variety of interest (recurrent parent) is crossed to a second variety (nonrecurrent parent) that carries the single gene of interest to be transferred. The resulting progeny from this cross are then crossed again to the recurrent parent and the process is repeated until a potato plant is obtained wherein essentially all of the desired morphological and physiological characteristics of the recurrent parent are recovered in the converted plant, in addition to the single gene or a limited number of genes transferred from the nonrecurrent parent.

The selection of a suitable recurrent parent is an important step for a successful backcrossing procedure. The goal of a backcross protocol is to alter or substitute a single trait or characteristic in the original variety. To accomplish this, a single gene of the recurrent variety is modified, substituted or supplemented with the desired gene from the nonrecurrent parent, while retaining essentially all of the rest of the desired genes, and therefore the desired physiological and morphological constitution of the original variety. The choice of the particular nonrecurrent parent will depend on the purpose of the backcross. One of the major purposes is to add some commercially desirable, agronomically important traits to the recurrent parent. The exact backcrossing protocol will depend on the characteristic or trait being altered or added to determine an appropriate testing protocol. Although backcrossing methods are simplified when the characteristic being transferred is a dominant allele, a recessive allele may also be transferred. In this instance, it may be necessary to introduce a test of the progeny to determine if the desired characteristic has been

successfully transferred. Preferably, such genes are monitored by diagnostic molecular markers.

Likewise, transgenes can be introduced into the plant using any of a variety of established recombinant methods well-known to persons skilled in the art.

Many single gene traits have been identified that are not regularly selected for in the development of a new variety but that can be improved by backcrossing and genetic engineering techniques. Single gene traits may or may not be transgenic, examples of these traits include but are not limited to: herbicide resistance; resistance to bacterial, fungal or viral disease; insect resistance; uniformity or increase in concentration of starch and other carbohydrates; enhanced nutritional quality; decrease in tendency of tuber to bruise; and decrease in the rate of starch conversion to sugars.

The term "selfing" refers to the process of self-fertilization wherein an individual is pollinated or fertilized with its own pollen. Repeated selfing eventually results in homozygous offspring.

A "line", as used herein, refers to a population of plants derived from a single cross, backcross or selfing. The individual offspring plants are not necessarily identical to one another. It is possible that individual offspring plants are not vigorous, fertile or self compatible due to natural variability. However, it is foreseen that suitable plants that are vigorous, fertile and self compatible can be easily identified in a line and used for additional breeding purpose.

As used herein, the term "allele(s)" means any of one or more alternative forms of a gene, all of which alleles relate to at least one trait or characteristic. In a diploid cell or organism, the two copies of a gene occupy corresponding loci on a pair of homologous chromosomes. Each copy may be a distinct allele.

A "gene" is defined herein as a hereditary unit (often indicated by a sequence of DNA) that occupies a specific location on a chromosome and that contains the genetic instruction for a contribution to potential phenotypic characteristics or trait in a plant.

A "locus" is defined herein as the position that a given gene occupies on a chromosome of a given plant species.

As used herein, the term "homozygous" means a genetic condition existing when identical alleles reside at corresponding loci on homologous chromosomes.

The term "essentially homozygous" refers to a level of homozygosity of at least 25%, preferably at least 50%, more preferably at least 75%, still more preferably at least 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% homozygosity when testing 100, preferably 1000, more preferably at least 10.000 loci.

Homozygosity levels are average values for the population, and refer preferably to those loci wherein the parents differ.

As used herein, the term "heterozygous" means a genetic condition existing when different alleles reside at corresponding loci on homologous chromosomes.

The term "recombination" or "recombine" refers to the exchange of information between two homologous chromosomes during meiosis. In a "recombinant" plant, DNA that is originally present on a specific location within the chromosome, e.g. linked to a gene/locus, is exchanged for DNA from another plant (i.e. maternal for paternal or *vice versa*). In order to exchange only the required material, and maintain the valuable original information on the chromosome as much as possible, two flanking crossover or recombination events will usually be required. In a double recombinant this exchange has taken place on both sides of a gene/locus. one way to find such a double recombinant, is to screen a population of F2-plants. This population has to be large, since double recombination occurs in only a limited frequency. Alternatively, double recombinants within a genetic unit can be the result of subsequent backcrossing. The frequency of double recombination is the product of the frequencies of the single recombinants. (E.g. a recombinant in a 10 cM area can be found with a frequency of 10%, double recombinants are found with a frequency of 10% x 10% = 1 %).

As used herein, the term "progeny" means (a) genetic descendant(s) or offspring.

As used herein, the term "population" means a genetically heterogeneous collection of plants sharing a common genetic derivation.

A "recombination event" refers to a mitotic or meiotic crossing-over event, including a GMO event.

As used herein, the term "hybrid" means any offspring of a cross between two genetically unlike individuals, more preferably the term refers to the cross

between two (elite or inbred) breeding lines which will not reproduce true to the parent from seed.

The term “segregate”, as used herein, refers to the separation of paired alleles during meiosis so that members of each pair of alleles appear in different gametes. The term includes reference to the result of this genetic phenomenon wherein the offspring population of a crossing in which at least one of the parents is heterozygous for an allelic gene is non-uniform with respect to phenotypic trait conferred by said gene.

The term “breeding line”, as used herein, refers to a line of a cultivated potato having commercially valuable or agronomically desirable characteristics, as opposed to wild varieties or landraces. The term includes reference to an elite breeding line or elite line, which represents an essentially homozygous, usually inbred, line of plants used to produce commercial F_1 hybrids. An elite breeding line is obtained by breeding and selection for superior agronomic performance comprising a multitude of agronomically desirable traits. An elite plant is any plant from an elite line. Superior agronomic performance refers to a desired combination of agronomically desirable traits as defined herein, wherein it is desirable that the majority, preferably all of the agronomically desirable traits are improved in the elite breeding line as compared to a non-elite breeding line. Elite breeding lines are essentially homozygous and are preferably inbred lines.

The term "elite line", as used herein, refers to any line that has resulted from breeding and selection for superior agronomic performance. An elite line preferably is a line that has multiple, preferably at least 3, 4 5, 6 or more (genes for) desirable agronomic traits as defined herein.

The terms "cultivar" and “variety” are used interchangeable herein and denote a plant with has deliberately been developed by breeding, cq crossing and selection, for the purpose of being commercialized, cq used by farmers and growers, to produce agricultural products for own sonsumption or for commercialization (fresh consumption, processing, feed, etc). The term “breeding germplasm” denotes a plnat having a biological status other than a "wild" status, which "wild" status indicates the original non-cultivated, or natural state of a plant or accession.

The term "breeding germplasm" includes, but is not limited to, semi-natural, semi-wild, weedy, traditional cultivar, landrace, breeding material, research

material, breeder's line, synthetic population, hybrid, founder stock/base population, inbred line (parent of hybrid cultivar), segregating population, mutant/genetic stock, market class and advanced/improved cultivar. Examples of cultivars include such cultivated varieties as Bintje, Russet Burbank, Eigenheimer, and Nicola.

As used herein, the terms "purebred", "pure inbred" or "inbred" are interchangeable and refer to a substantially homozygous plant or plant line obtained by repeated selfing and-or backcrossing.

As used herein, the term "molecular genetic marker" or short "marker" refers to an indicator that is used in methods for visualizing differences in characteristics of nucleic acid sequences. Examples of such indicators are restriction fragment length polymorphism (RFLP) markers, amplified fragment length polymorphism (AFLP) markers, single nucleotide polymorphisms (SNPs), insertion/deletion (INDEL) mutations, microsatellite markers (SSRs), sequence-characterized amplified regions (SCARs), cleaved amplified polymorphic sequence (CAPS) markers or isozyme markers or combinations of the markers described herein which defines a specific genetic and chromosomal location.

As used herein, the term "plant part" indicates a part of the potato plant, including organelles, single cells and cell tissues such as plant cells that are intact in plants, cell clumps and tissue cultures from which potato plants can be regenerated. Examples of plant parts include, but are not limited to, single cells and tissues from pollen, ovules, leaves, embryos, roots, root tips, tubers, anthers, flowers, fruits, stems shoots, and seeds; as well as pollen, ovules, leaves, embryos, roots, root tips, anthers, flowers, fruits, stems, shoots, scions, rootstocks, seeds, tubers, protoplasts, calli, and the like.

Unless expressly stated otherwise, the term "seed", as used throughout this specification, refers to the body from which a new plant develops (or kernel in some plants) containing the small embryonic plant enclosed in a seed coat covering, usually together with some stored food. This seed, referred to as botanical or "true" seed is the product of the ripened ovule of gymnosperm and angiosperm plants which occurs after fertilization and some growth within the mother plant.

As used herein the terms "vigour" and "vigorous" refer to the relative amount of above-ground or below-ground tissues of a plant, which relative amount may be more or less independent of one another.

The relative amount of above-ground tissues can be expressed as visual observations of the amount of foliage, in terms of plant height, number of stems, number of leaves assessed on ordinal scales, and/or in terms of fresh weight or dry matter. This trait has relevance during the first period of the plant growth cycle with an average duration of 100 days of cultivation (range 75 – 130 days) under normal growth conditions that prevail in North Western Europe during the season from May trough to August. Under these conditions, the plants of the present invention attain a weight of the foliage and shoots (above-ground plant) expressed in grams of fresh or dry weight which is at least 50%, more preferably at least 60%, 70%, 80%, 90% or more of the (essentially heterozygous) diploid breeding clone RH89-039-16, which – in turn - has 68% (range 44 – 86%) of the tuber yield of tetraploid potato varieties based on nine years of testing. Clone RH89-039-16 is available for reference purpose from Dr. Ir Ronald B.C. Hutten, Laboratory of Plant Breeding, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.

Alternatively, or in addition, the term vigour may be used in connection with a certain minimal tuber yield. The term "tuber yield", as used herein, will generally refer to a tuber yield expressed in grams of fresh weight which at harvest in plants according to the present invention is at least 30-50%, more preferably at least 60%, 70%, 80%, 90% or more of the (essentially heterozygous) diploid breeding clone RH89-039-16, which – in turn - has 68% (range 44 – 86%) of the tuber yield of tetraploid potato varieties based on nine years of field testing under conditions of a 100 days cultivation period with normal growth conditions that prevail in North Western Europe during the season from May trough to August. As indicated above, clone RH89-039-16 is available for reference purpose from Dr. Ir Ronald B.C. Hutten, Laboratory of Plant Breeding, Wageningen University.

Tuber yield, unless expressly stated otherwise, is based on the tubers formed from a seedling plant, in contrast to the tubers formed from a tuber-grown plant – which yield may be 50-200% more than the yield of a seedling.

Alternatively, or in addition, the term vigour may refer to a tuber yield expressed in grams of fresh or dry weight which at harvest is at least 30-50%, more preferably at least 60%, 70%, 80%, 90% or more of the diploid breeding clone RH89-039-16. This means that the tuber yield expressed in grams of fresh weight may (in plants of the present invention, reach levels of at least 200, more preferably at least

300, 400 or 500 grams per plant grown from seed in clay soil under normal North Western European summer conditions, and even 400, 500, 600, 700, 800 or more, such as 900 or 1000 grams of fresh tuber weight or more per plant when said plant is raised from tubers and grown in clay soil under normal North Western European summer conditions.

The term "a line comprising plants", wherein said plants are indicated as having a certain characteristic, such as tuber yield, should preferably be understood as referring to a line essentially consisting of plants having the said trait, while allowing for some biological variation.

The term "diploid" as used herein refers to a plant wherein each vegetative cell contains two sets of chromosomes (2x=2n, wherein n is the number of chromosomes). One set of chromosomes is donated from each parent.

The term "tetraploid" as used herein refers to a plant wherein each vegetative cell contains four sets of chromosomes (2x=4n).

The term "nematode resistance" as used herein refers to a plant in which a functional resistance gene has been introgressed that prevents the multiplication of at least one nematode population or isolate.

The term "disease resistance" as used herein refers to the ability to show more than 50 % reduction of the amount of diseased leaf surface or tuber volume, or the amount of multiplication of an insect or pathogenic microorganism including but not limited to *Streptomyces* spp, *Rhizoctonia*, Silver scurf and, *Phytophthora infestans*,

The term "herbicide tolerance" as used herein refers to a plant which shows less than 50% damage of the foliage than the usual damage upon the application of a specified dosage of herbicide.

The term "cold tolerance" as used herein refers to the average amount of leaf surface of a plant which shows frost damage, which damage in cold tolerant plants is less than the average damage observed at a temp of minus 3 °C for relatively cold sensitive reference varieties such as e.g. Caribe and/or Kennebec.

The term "drought tolerance" as used herein refers to a plant which shows less than average damage at limiting water conditions as compared to relatively drought sensitive reference varieties such as Caribe and/or Carlton.

The term "flooding tolerance" as used herein refers to a 50% lower proportion of affected tubers (anaerobic degradation) relative to a plant which shows average damage at flooding

The term "tolerance to wet rot" as used herein refers to a plant with the slightest level of resistance to *Erwinia* species (currently termed *Pectobacterium* spp.).

The term "tolerance to dry rot" as used herein refers to a plant with the slightest level of resistance to *Fusarium* species.

The term "salinity tolerance" as used herein refers to a plant which shows less damage than average at salinity conditions.

The term "growth rate" as used herein refers to the increase of plant biomass per unit of time.

The term "tuber development defects" (e.g. misshapen or damaged tubers)" as used herein refers to a plant which shows visual as well as internal malformations of tubers during plant development and/or tuber harvest.

The term "tuber yield" as used herein refers to the total weight of tubers of a plant or of a population of plants generally expressed in grams of fresh weight.

The term "tuber size" as used herein refers to the absolute width, height and length of a tuber. Beneficial tuber sizes include plant- average tuber sizes of about 80-160 grams per tuber. Mean tuber number for commercially valuable plants are about 8-12 tubers per plant.

The term "tuber skin colour" as used herein refers to the colour of the skin of a tuber after harvest as the result of anthocyanin accumulation in tuber skin tissues.

The term "eye depth" as used herein refers to the relative distance between the skin surface and the shoot primordium of a tuber.

The term "tuber shape" as used herein refers to the length / width ratio, to indicate the continuous variation from round, oval to long tuber shaps, as well as the height/width ratio, to indicate the continuous variation from cylindrical to the amount of flatness of a tuber.

The term "tuber flesh colour" as used herein refers to the colour of the interior of the tuber flesh after harvest as the result of the absence or presence of carotenoid compounds causing white or yellow flesh colour, respectively, as well as the

absence of anthocyanins compounds causing red, blue, purple shades of flesh colour, irrespective of the presence of colour in patterns being partial or full.

The term "tuber taste" as used herein refers to the consumers appreciation by consuming a cooked potato tuber.

The term "tuber shelf life" and "tuber storage ability" as used herein are synonymous and refer to lack of changes in appearance of a tuber at storage.

The term "tuber dormancy period" as used herein refers to the time period between sowing and sprouting of a tuber, while sown at normal conditions.

The term "resistance to tuber dehydration" as used herein refers to a plant with shows less dehydration of the tuber than average at low humidity conditions (see shelf life).

The term "tuber starch content" as used herein refers to the starch weight over the total fresh weight of a tuber.

The term "tuber dry matter content" as used herein refers to the weight of the dry components of a potato divided over the total fresh weight.

The term "tuber cooking quality" as used herein refers to the continuous variation from firm to mealiness of a tuber after cooking.

The term "tuber frying quality" as used herein refers to the consumers appreciation of a tuber after frying.

The term "tuber chip making quality" as used herein refers to the consumers appreciation of a tuber after making chips.

The term "tuber uniformity" as used herein refers to the standard deviation of tuber traits, whereby a low standard variation refers to high uniformity and vice versa.

The term "cold sweetening resistance" as used herein refers to resistance of a tuber to produce glucose and/or fructose while stored at temperatures below 8 °C.

The term "tetraploid" as used herein refers to a plant wherein each vegetative cell contains four sets of chromosomes (4n). The terms "fertile" and "fertility" broadly refer to the capacity to reproduce, i.e. to conceive and to produce offspring, preferably fertile offspring. Thus, the term fertility refers to individuals that can be crossed to produce fertile offspring in that the flowers, when pollinated produce berries that contain seeds which when sown result in a plant that itself carries fertile flowers, etc. .

The term "fertile offspring" or "fertile seed" is defined herein as seed capable of growing into a flower-producing potato plant wherein flowers are male and female fertile. Thus, the term preferably refers to a plant or seed that when grown into a plant is capable of producing offspring as male and as female parent by virtue of the presence of fertile ovules and fertile pollen (i,e, both male and female flowers are fertile).

The term "fertile plant" is defined herein as a plant capable of producing fertile seed carrying berries. Preferably said berries each carry at least 5, more preferably at least 15-20, even more preferably at least 50 fertile seed, still more preferably between 50 and 500 seeds. Alternatively, a plant is said to be fertile when the number of seeds produced per berry is at least 10%, more preferably at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150% 200% or more of the number found in berries of the diploid breeding clone RH89-039-16. The term also, or alternatively, refers to plant lines having more than 25 %, preferably more than 90% fertile plants.

The term "self-compatible" refers to capacity to develop seeds in berries that are the result of self-pollination, self fertilization and producing fertile progeny.

The term "capable of being backcrossed/selfed/crossed and resulting in (self) fertile and self-compatible offspring" refers to the capability of producing one or more berries with seed as a result of backcrossing, selfing or crossing and wherein more than 60%, preferably more than 70%, 80%, 85%, 90% or more of said seed in said one or more berries is capable of developing into plants that are (self) fertile and self-compatible.

For other terms as used herein, reference is made to Allard, R. W. Principles of Plant Breeding, 2nd Edition, Wiley New York, 1999, and specifically to the Glossary therein.

Description of the preferred embodiments

The present inventors discovered that elite breeding lines for potato breeding can be successfully produced. Crossing of two elite breeding lines provides F1 hybrid seeds, such F1 hybrid seeds, when grown into plants, result in plants of superior agronomic performance. More importantly, all seeds are genetically essentially identical, meaning that, except for the occasional crossover or mutation event, all seeds are uniform.

The present inventors have discovered a method that allows the provision of elite potato breeding lines, which, when crossed mutually, provide uniform F1 hybrid potato seeds which, when grown into plants, result in plants having superior agronomic performance. The elite potato breeding lines may have complementary traits and, as a result of their homozygosity, the F1 plants grown from the F1 hybrid seeds are uniform, but when these F1 plants are subsequently selfed or cross-pollinated the resulting F2 plants will segregate for the various traits.

Thus, in contrast to the currently available true potato seeds (TPS) generated in the conventional ways of the prior art and harvested from berries in which every individual seed is genetically different from every other seed, the seeds obtained from crossing of the potato lines and elite potato breeding lines in accordance with aspects of the present invention are genetically essentially identical.

The TPS varieties of the prior art are derived from crosses between selected but heterozygous parents and hence variability is expected in plants and tubers. Hybrid potato breeding as proposed herein differs from the conventional production of TPS in that the present TPS are the result of the crossing of two pure breeding lines, resulting in uniform cultivars, whereas TPS varieties of the prior art are the result of open pollination or crossing between two genetically non-homozygous and often tetraploid breeding lines resulting in non-uniform cultivars.

Not only are the presently provided plants novel in that they combine a number of properties, heretofore never attained together in a single potato line, they also allow for novel and very advantageous breeding methods. Hence, the plants of the invention are an intermediate in the production of novel potato cultivars, which cultivars can be produced by methods as disclosed herein. The advantageous breeding methods allow for the elimination of many of the drawbacks of conventional potato breeding as indicated in hereinabove including the elimination of the time-consuming selection of clones to arrive at a new potato variety and the possibility to provide uniform hybrid potato seed. In addition, the novel plants and methods also allow for the elimination of drawbacks related to clonal propagation and production, including the possibility to eliminate high transportation costs and the clonal persistence of pests and diseases.

Novel potato plants

Parental breeding lines

The present invention provides potato plants that are essentially homozygous, diploid, vigorous, fertile and self-compatible.

Preferably the plants of the invention have fixed within their genome nucleic acid sequences encoding for genes that confer to the plant an agronomically desirable trait. Such nucleic acid sequences may be introgressions or transgenes derived from potato germplasm with agronomically desirable traits, preferably from diploid germplasm.

- *Essentially homozygous*

In breeding, plants are propagated by sexual reproduction, usually by crossing of selected parents with desirable traits to produce improved progeny. Progeny inherit genes for both desirable and undesirable traits from both parents. Repeated selection of meritorious individuals from each generation that will serve as parents of the next will conserve desired characteristics and eliminate undesirable ones. The ultimate parent in plant breeding is a purebred, a highly homozygous line that, when used in crossings with other purebreds results in offspring that is identical, both phenotypically and genotypically, within a single generation and between consecutive generations obtained from other crossings of the same parents. Purebreds, or pure inbreds, can be obtained by everal generations of selfing or inbreeding (crossing of close relatives, such as brother to sister plants or offspring to parent (backcrossing)) each successive generation showing an increased degrees of genetic uniformity, or homozygosity. The use of homozygous inbreds as breeding parents results in offspring that is identical, both phenotypically and genotypically, within a single generation and between consecutive generations obtained from other crossings of those parents. Hence, the pure inbreds can be used repeatedly to produce the same offspring plants.

In potato, selfing and inbreeding results in severe inbreeding depression, or the expression of deleterious recessive genes, which expression is revealed by loss of vigour and loss of fertility. Hence, it has hitherto been impossible to produce homozygous pure inbreds in potato since until the present invention, high levels of homozygosity in potato with sufficient vigour could not be attained. The present invention now, for the first time, provides plants having a level of homozygosity that

is above 25%, and preferably above 75 % and that is combined with fertility, self compatibility and plant vigour.

- *Diploid*

The plants of the present invention are diploid plants, in contrast to the commercial potato varieties, which are tetraploid. It should be noted that in plants of the present invention higher ploidy levels are expressly envisaged, since the diploid stage is used primarily during the development of the breeding lines. Once the purebreds have been established, the lines may be made tetraploid. Hence, plants of the invention may also be tetraploid. Tetraploid plants can be produced from tetraploid cells or tissue cultures, which in turn may be produced by duplicating the genome of diploid cells or tissue cultures by methods known per se, for instance by using oryzalin as described by Barandalla *et al.* (Barandalla et al. Potato Research (2006) 49:143-154). Tetraploid plants may have the advantage that they provide higher yields.

- *Vigorous*

Although commercial tetraploid potato varieties are fertile and self-compatible and vigorous in that they grow well, do not produce deformed plants, or fruits, but manifest as healthy plants with a high tuber yields, their selfed progeny shows serious inbreeding depression, that is, result in faint growing and low yielding plants with deformed tubers. Prior art diploid and essentially homozygous lines, such as those reported by Phumichai et al. as referred to herein, also grow only very poorly, and manifest as very weak and small plants, that have poor seed set. In fact, diploid potato plants resulting from inbreeding, and attaining homozygosity of at least 25 % or 50% on the alleles are invariably weak growers.

The plants of the present invention are not weak and frail, and although they need not necessarily be as vigorous as present day commercial tetraploid varieties, they do not show inbreeding depression. Hence, within the context of the present invention, they are vigorous. Tuber formation exemplifies the vigour of the plants of the present invention. Although all commercial (tetraploid and seed-tuber derived) potato varieties produce large tubers, or at least tubers of sufficient size, prior art diploid lines and essentially homozygous plants produce only small tubers,

which are, for instance, of no use in the production of potato chips or French fries. Prior art diploid lines and essentially homozygous plants produce also very few tubers such that the yield of tubers per plant is at least ten times lower than that of heterozygous diploids plants and commercial tetraploid potato varieties. In contrast, the plants of the present invention exhibit beneficial tuber formation characteristics, including tubers of beneficial size profile, and a tuber yield that may be at least 50% compared to the diploid heterozygous plants used as donors in the examples as described herein (D1, D2, and/or D3).

As was found herein, the tuber yield of the diploid F1's was on average 443 grams per plant, with values as high as 600 and 634 grams.

Backcrossing with the F1: "IVP08-06" yielded on average 155 and 209 grams, while backcrossing with the F1: "IVP08-07" yielded on average 209 and 259 grams.

The "pseudo F2's" yielded on average 172 and 332 grams per plant, and occasionally produced higher than the diploid F1's.

The F2's yielded on average 69 and 252 grams per plant. Several plants overlap with the yield values of the diploid F1s.

- *fertile*

The plants of the present invention – although essentially homozygous – are also fertile. That means that they can be used in breeding programs because their offspring will also be fertile.

- self compatible

The plants of the present invention are self compatible. This feature is introduced in order to allow the selfing of plants and the production of homozygous lines. The preferred basis of the self compatibility is derived from the dominant S locus inhibitor gene (*Sli*) (Hosaka and Hanneman, 1998, Euphytica, 99: 191–197).

The essentially homozygous potato plants of the present invention have as an advantage that they can be crossed with other essentially homozygous potato plants whereby the hybrid offspring of said cross is essentially genetically uniform and will not segregate.

The present invention, in a preferred embodiment, provides essentially homozygous, diploid, vigorous, fertile, and self-compatible potato plants having agronomically desirable traits.

The novel potato plants of the invention are capable of:

- being backcrossed to an essentially homozygous recurrent parent and resulting in (self) fertile and self-compatible offspring;
- being selfed (self-fertilization of 1 offspring individual) and resulting in (self) fertile and self-compatible offspring;
- being mutually crossed (fertilization between 2 offspring individuals) and resulting in (self) fertile and self-compatible offspring.

Plants according to the present invention include the line with breeders reference AGVD1, representative seeds of said line having been deposited on 23 October 2009 with the NCIMB, Aberdeen, Scotland under accession number NCIMB 41663 and breeders reference AGVD1, which plant represents an F2 obtained by crossing a plant of parent IVP07-1001/4 (see Example 1), which is an essentially homozygous, diploid, fertile and self-compatible but non-vigorous potato line with a plant of parent D1, which is an elite diploid breeding clone that is essentially non-homozygous, and is not self-compatible, but which has the desirable agronomic trait of yellow flesh color and exhibits good cooking quality in that the flesh is not greying after cooking, and selfing the resulting F1 offspring plants. It is estimated that AGVD1 is about 50% homozygous, meaning that about 50% of the loci for which the parents differ are homozygous.

Plants according to the present invention also include the line with breeders reference AGVD2, representative seeds of said line having been deposited on 23 October 2009 with the NCIMB, Aberdeen, Scotland under accession number NCIMB 41664 and under breeders reference AGVD2, which line represents an (F1 x F1) line (or pseudo F2) obtained by crossing a plant of parent IVP07-1001/4 as indicated above with a plant of parent D1 as indicated above, and mutually crossing individual F1 offspring plants, that share the IVP07-1001/4 parent but have different individuals of the D1 parent. It is estimated that AGVD2 is about 25% homozygous for the loci for which the parents differ.

Plants according to the present invention also include the line with breeders reference AGVD3, representative seeds of said line having been deposited on

23 October 2009 with the NCIMB, Aberdeen, Scotland under accession number NCIMB 41665 and under breeders reference AGVD3, which line represents a BC1 population obtained by crossing a plant of parent IVP07-1001/4 as indicated above with a plant of parent D1 as indicated above, and backcrossing the resulting F1 offspring plants with the IVP07-1001/4 parent. It is estimated that AGVD3 is about 75% homozygous for the loci for which the parents differ.

Plants according to the present invention also include the line with breeders reference AGVD17, representative seeds of said line having been deposited on 5 October 2010 with the NCIMB, Aberdeen, Scotland under accession number NCIMB 41765 and under breeders reference AGVD17, which line represents an F3 population obtained by crossing a plant of parent IVP07-1001/4 as indicated above with a plant of parent D2 as indicated above, and backcrossing the resulting F1 offspring plants with the IVP07-1001/4 parent, followed by two steps of selfing. It is estimated that AGVD17 is > 80% homozygous for the loci for which the parents differ.

A plant of the present invention can very suitably be used as a recurrent parent wherein additional agronomically desirable traits are introgressed by crossing, or introduced by other means such as by transgenic means. Once a number of agronomically desirable traits have been stacked into an essentially homozygous, vigorous, diploid, fertile and self-compatible plant according to the invention, that plant can be used as an elite breeding line for the production of commercial F_1 hybrid potato seed. Likewise, a second elite breeding line can be provided that can serve as the parent of opposite sex so that both plants can be crossed to produce the commercial F_1 seed of a novel potato variety.

The breeding of uniform hybrid seed depends on a number of technical conditions. Prerequisites to achieve uniform hybrid seed are that i) an initial introgression crossing can be made between an essentially homozygous, diploid, fertile and self-compatible plant and a plant that acts as donor for an agronomically desirable trait, that ii) a backcross can be produced between the offspring of that initial crossing and the essentially homozygous, diploid, fertile and self-compatible plant, and that iii) the offspring thereof can be selfed. Potato lines AGVD1, AGVD2 and AGVD3 exhibit these characteristics and individual plants of these lines can be used in additional introgression crossings, backcrossings and selfings to arrive at the

elite breeding lines suitable for use as parents for commercial F1 hybrid seed that can be grown into hybrid potato plant of a new potato variety.

A highly preferred plant according to the present invention is a plant of line AGVD2 or an offspring plant thereof having all of the physiological and morphological characteristics of the plant of line AGVD2.

Another line deposited as example of embodiments of the present invention is AGVD17 (NCIMB 41765, deposited 05 October 2010). The advantage of AGVD17 is that it is a highly homozygous line (> 80% homozygous) combining vigour and fertility with a tuber yield of on average 300 grams (fresh weight) per plant. Individual plants of related populations even showed tuber yields of 500 grams per plant. These data were generated from field grown plants that were raised from seedlings. Potato plants raised from clones generally yield tuber weights that are 50 to 100% higher than plants raised from seedlings. Hence, the tuber yield expressed in grams of fresh weight from plants as claimed herein – when clonally propagated and raised from tubers and grown in clay soil under normal North Western European summer conditions – is expected to reach levels of at least about 1000 grams fresh weight per plant.

Thus, line AGVD17 demonstrates in particular the possibility to intercross individual elite lines of the present invention (i.e. that are self-compatible, fertile, and essentially homozygous) to arrive at the hybrid seed that is the aim of the present invention. Hence, another highly preferred plant according to the present invention is a plant of line AGVD17 or an offspring plant thereof having all of the physiological and morphological characteristics of the plant of line AGVD17.

Hybrids

The hybrid plants of the invention are produced by a method of the invention as exemplified in Figure 1. The hybrid plants of the invention are produced by crossing two essentially homozygous elite potato parental breeding lines comprising among them a number of agronomically desirable traits selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity

tolerance, cold sweetening resistance and resistance to tuber dehydration. In addition to these agronomically desirable traits, the parental breeding lines may exhibit such characteristics as desired in specific use markets such as desirable tuber skin color, desirable tuber shape, desirable tuber flesh color, desirable tuber taste, desirable tuber starch content, desirable tuber dry matter content, desirable tuber cooking quality, or desirable tuber frying quality. In addition to such market-specific characteristics, the parental breeding lines, when crossed, preferably result in hybrids that exhibit such beneficial traits as beneficial growth rate, beneficial tuber yield, beneficial tuber size, beneficial tuber storage ability, beneficial tuber dormancy period, beneficial tuber shelf life, beneficial tuber chip making quality, and beneficial tuber uniformity.

The present invention now provides a set of potato plants or a set of potato plant lines, which plants or plant lines are: diploid (2n); essentially homozygous (preferably more than 60% on all alleles); self compatible; fertile; and vigorous. Hitherto it has proven impossible to provide the combination of all these features in a single potato plant (line). The set of potato plant of the invention can be used to produce novel varieties or cultivars.

The invention particularly relates to a set of at least 2 diploid (2n), essentially homozygous (preferably more than 60% on all alleles), self compatible, fertile, and vigorous plants that are crossable to produce hybrid varieties according to the invention.

Novel method of potato seed breeding

The provision of the novel potato plants now provides for the first time the attainment of the method of the invention.

The present inventors have generated potato plants that are essentially homozygous, diploid, vigorous, fertile and self-compatible and having fixed within their genome nucleic acid sequences encoding genes that confer to the plant an agronomically desirable trait derived from suitable diploid donor lines. The said nucleic acid sequences are the result of introgressions from potato germplasm having agronomically desirable traits. Conventionally, at least in most species other than potato, homozygous plants can in principle be obtained in as little as two generations by doubling of monohaploids generated from unfertilised generative cells such as egg

or pollen cells by methods known as doubled haploid (DH) technology. This is not possible in potato, as the resulting plants have hitherto shown to be infertile, due to - what is considered – expression of recessive deleterious genes. The DH method was extensively tested by the present inventors, but was found unsuccessful.

The present inventors used the strategy of repeated selfing, backcrossing and crossing of potato germplasm of a single self-compatible potato line and one or more self-incompatible potato lines harbouring agronomic traits. The homozygous plants of the present invention were obtained by continuous selfing of diploid potato plants and selection for fertile offspring (besides the inherent selection for self-compatibility). This is an entirely new approach, as in the prior art methods, plants are obtained by selection for agronomical traits, such as tuber formation or general growth characteristics of the offspring plants, not by selection for fertile offspring from selfings. Moreover, and unlike the prior art methods the procedures are performed using diploid plant lines, instead to tetraploid plants. Unexpectedly, this radically new approach proved successful for obtaining increasingly homozygous, diploid offspring plants, that were self-compatibel and fertile. In addition, when (out)crossed with plants of diploid lines harbouring beneficial traits, such as resistance or white tuber color, these traits could be introduced into the essentially homozygous germplasm without loss of self-compatibility and fertility.

In essence the method for obtaining the plants is as follows:

Starting point is a diploid self-compatible potato line (examples of suitable lines have been deposited under depositors reference AGVD 1, AGDV 2, AGDV 3 and AGVD17). In general a line being self-compatible due to the presence of the *Sli* gene may be used as starting point. Plants of this diploid self-compatible line are selfed and seeds obtained from seed-carrying berries are sown and grown into offspring plants. Offspring plants carrying flowers are again selfed and seeds obtained from seed-carrying berries are again sown and grown into offspring plants. No, or at least little, attention is paid to the agronomical appearance of the plants at this stage. They may look withered and frail, with only a few flowers. Nonetheless, the selfing is repeated at least three times, but preferably 4 or 5 or even 6-8 times. In this manner a line of essentially homozygous, fertile, self-compatible, and diploid potato plants is obtained, essentially by selfing and selecting for plants that produce fertile offspring.

Next, an agronomically desirable trait is added to the essentially homozygous, fertile, self-compatible, diploid potato genotype by crossing a plant of that line with a plant of an unrelated essentially heterozygous diploid potato line, preferably a line containing an agronomically desirable trait. This essentially heterozygous line (which term merely reflects that the line is not an essentially homozygous line as used herein) functions as a donor for an agronomically desirable trait, in this case vigour.

The resulting hybrid is selfed and/or backcrossed to the self-compatible line while selecting for higher vigour. In this way, the vigour of the essentially homozygous, fertile, self-compatible, diploid potato plant line is enhanced and at the same time the line is agronomically improved. These steps will thus result in a vigorous fertile homozygous self-compatible diploid potato: The vigour may further be enhanced by crossing with other essentially heterozygous diploid potato lines, which additional lines serve as donors for additional agronomically desirable traits, followed by selfing and/or backcrossing to to the self-compatible line while selecting for these additional traits, which additional steps will result in the provision of diploid potato breeding lines.

In order to produce elite diploid potato breeding lines, the breeding value of the breeding lines is further enhanced by intercrossing, selfing and backcrossing while continuous selecting for the agronomically desirable traits provided to the breeding line, and by establishing lines that exhibit good combining abilities with other lines. Such combining abilities are evaluated by the use of experimental crosses.

Finally, hybrid potato cultivars can be produced by crossing individual elite diploid potato breeding lines that exhibit good combining abilities. The seeds resulting from this cross of homozygous parents are hybrid true potato seeds that are uniform.

Using the methods of the present invention, generally, around 7 to 8 selfing cycles will result in essentially homozygous plants, but the skilled artisan will understand that more generations of selfing may also be used. Also fewer generations, such as six generations may be used to provide essentially homozygous plants of the present invention. The number of backcross generation can even be reduced to three to reach more than 95% homozygosity when applying molecular genetic markers.

Essentially, the novel method of producing hybrid potato cultivars provides for the possibility to produce a new potato variety within about 5 years. A detailed process for the production of hybrid potatoes starting from AGVD17 (F3 line) is provided in Example 5. This line is >80% homozygous such that very few selfings are needed to obtain a plant that is about 95%-100% homozygous. In addition AGVD17 is already vigorous as defined herein.

The present invention now provides a method for producing a genetically homogenous or uniform true potato seed (TPS). This method is *inter alia* useful in a method of preventing transmittal of virus infections between potato generations, but also for overcoming many of the other problems associated with classical potato breeding, "seed" potato production and transport and cultivation. Moreover, the present parental material for production of hybrid seed can be maintained in the form of seed, and not in the form of vegetative material as is required by traditional TPS production.

The present invention provides in one aspect a method for producing a hybrid potato cultivar comprising the use of the deposited plant material provided herein as a starting material.

Alternatively, other essentially homozygous, diploid, fertile, vigorous and self-compatible plant lines different from the deposits referred to herein may be developed and the production of a hybrid potato cultivar can commence from that early stage level by using the teaching provided herein since the present inventors have demonstrated that this route, although laborious, is successful. Such an alternative breeding material is also vigorous, essentially homozygous, diploid, fertile and self-compatible.

As a first step, such an early stage development may comprise the provision of a plant of a first cultivated potato species (*Solanum tuberosum*) or a related crossable species (generally a tuber bearing *Solanum* species).

Next, said plant is developed into a line of self-compatible diploid potato plants, preferably a *Solanum tuberosum*. Diploid potato is usually self incompatible. Self-compatibility may suitably be introduced from related species (see for instance Hermsen, J.G.Th. 1978, Euphytica 27, 1-11).

The resulting line of self-compatible diploid potato plants is then developed into a homozygous fertile self-compatible diploid potato line. Homozygous diploids can

be obtained by doubling haploids (Uijtewaal et al., 1987, Theor. Appl. Genet. 73, 751-78) by anther culture (Jacobsen and Sopory, 1977, Theor. Appl. Genet. 52, 119-123), by ovule culture or by repeated selfings (Phumichai & Hosaka, 2006, Euphytica 149, 251-258). The latter method was used for producing the homozygous fertile self-compatible diploid potato line that formed the basis of the vigorous plants as described herein.

Next, the homozygous fertile self-compatible diploid potato line is developed into a vigorous fertile homozygous self-compatible diploid potato line. The vigour may be enhanced by crossing with other diploid potato plants, selfing and backcrossing while selecting for higher vigour. It must be noted that high populations are needed and that even for some unrelated essentially heterozygous diploid potato lines that are used as donor of the agronomically desirable trait, very few self compatible plants may be found in a population of 1000 plants. Generally, such attempts are terminated, and only when about 10-50% of the offspring plants is self-compatible (such as in the case of D2 in Example 2 below) the donor line exhibits such good general combining ability that a successful introduction of the agronomical trait into the self-compatible lineage is foreseen.

Next, the vigorous, fertile, homozygous, self-compatible, diploid potato line is developed into a diploid potato breeding line. Most preferably, at least two separate vigorous fertile homozygous self-compatible diploid potato lines are produced and at least two distinct diploid potato breeding lines are developed. Such breeding lines comprise a high number of agronomically desirable traits. Agronomically desirable traits are introduced by crossing with other diploid potato plants, selfing and backcrossing while selecting for these traits. This breeding program may comprise testing for disease, such as whether the shoots or tubers are free of disease, or whether they are resistant for disease.

Next, the diploid potato breeding lines are developed into elite diploid potato breeding lines. This is accomplished by enhancing the breeding value by intercrossing, selfing and backcrossing while continuously selecting for agronomically desirable traits. At this stage experimental crosses are performed to evaluate general combining abilities. The diploid elite lines can now also be developed into tetraploid (4x) elite lines by methods known per sé, such as by chemical treatment with e.g. colchicine, or by "spontaneous" doubling of the genome using tissue culture.

Finally, potato hybrid cultivars are produced from these elite diploid or tetreploid potato breeding lines by crossing of good combining breeding lines. The seeds obtained are hybrid true potato seeds that are uniform.

The method of the invention for producing a potato hybrid cultivar comprises the steps of producing a diploid potato breeding line. Said method comprises the steps of:

(a) providing a first potato plant, which first potato plant is a plant of a diploid, self-compatible and essentially homozygous potato line, preferably a plant of a vigorous fertile homozygous self-compatible diploid potato line, more preferably a diploid potato breeding line;

(b) providing a second potato plant of a potato line that may have any level of ploidy, preferably diploid, and/or any level of homozygosity wherein said second potato plant serves as a donor for an agronomically desirable trait which trait is to be introgressed into said essentially homozygous line of said first potato plant or at least in an offspring plant resulting from a crossing between said first and second plant, wherein said trait is selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, rooting ability, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, growth rate, tuber development defects (e.g. misshapen or damaged tubers), tuber yield, tuber size, tuber skin color, eye depth, tuber shape, tuber flesh color, tuber taste, tuber storage ability, tuber dormancy period, tuber shelf life, resistance to tuber dehydration, tuber starch content, tuber dry matter content, tuber cooking quality, tuber frying quality, tuber chip making quality, tuber uniformity and cold sweetening resistance.

Following the provision of these two plants, the method then comprises the step of:

(c) cross-pollinating said first and second potato plant such that one of said parent plants produces seed-carrying fruits (berries) and collecting said seeds from said fruits to provide a hybrid offspring seed.

Seeds are then grown to plants and the plants that comprise the agronomically desirable trait from the donor plant are selected, either directly or following additional selfing and backcrossing steps, as is well known in the art of

plant breeding. In these methods markers may be used to assist in the breeding process.

The hybrid offspring seed is preferably grown into a plant that is subsequently backcrossed a plant to the recurrent parent line

The following steps are not essential to either of the above methods. However, these additional steps may either provide for the production of consumption tubers by growing the seeds of the invention and/or may provide for the production of new homozygous, self-compatible, diploid elite breeding lines that can serve as parents for the inclusion of yet further traits into an offspring generation. The plants of the invention are fertile and vigorous. These additional benefits are attained by the following further steps of:

(d) growing said offspring seed into a hybrid offspring potato plant, and

(e1) harvesting tubers from said hybrid offspring potato plant to provide tubers carrying said trait, or

(e2) selfing said hybrid offspring potato plant or backcrossing said hybrid offspring potato plant to a potato plant of said line of said first potato plant to provide further offspring plants carrying said trait and wherein said of offspring plants are increasingly homozygous following each backcrossing or selfing step.

Preferably said second potato plant used in the method described above is a hybrid offspring potato plant as defined in step (d) above and obtained by the method as described above. More preferably, said second potato plant used in the method described above is a hybrid offspring potato plant as defined in step (e2) above and obtained by the method as described above.

Preferably, said step (e2) comprises from 2 to 7 backcrosses using a plant of line AGVD1, AGVD2, AGVD3 or AGVD17 as the recurrent parent and testing offspring plants for the presence of the trait (e.g. in the form of the homozygous or heterozygous presence of alleles responsible for the trait), followed by one or two (or more) selfings, to provide an essentially homozygous plant that is genetically equal to a plant of population AGVD1, AGVD2, AGVD3 or AGVD17, but in which alleles responsible for the trait are heterozygously, preferably homozygously present. This plant has at least one additional allele not available in population AGVD1, AGVD2, AGVD3 or AGVD17.

The above method now provides a breeding process for the production of homozygous breeding lines or elite breeding lines based on the background of population AGVD1, AGVD2, AGVD3 or AGVD17, to which background successively additional alleles are added, thereby providing elite breeding lines wherein desirable traits are stacked.

The method of the present invention now for the first time provides for a method for preventing transmittal of virus infections between potato generations while at the same time also solving many of the other problems of the prior art related to breeding of new potato varieties and commercial production of potatoes for consumption. This has become possible through the provision of true potato seed, i.e. botanical seed, which seed can be grown to a potato plant that produces tubers for consumption, and which seeds are from a cross in which at least one of the parents is a plant of a diploid, self-compatible and essentially homozygous potato line, preferably said seeds are from a cross wherein both of the parents are plants from a diploid, self-compatible and essentially homozygous breeding line.

An important advantage of this new method is that, in this way, the transmittal of infection with a virus that is not spread through the gametes and seeds can effectively be prevented.

The present inventors now provide a method for further breeding for homozygous lines that are easy to handle, and which exhibit improved fertility and plant vigour when compared to prior art inbreds, while maintaining the self-compatibility and high level of homozygosity. It will be appreciated that – just as in tomato - a 100% homozygosity is not always needed to provide for an effective breeding program. This is not a problem as long the self-compatibility is maintained.

The genetic transfer of agronomically desirable or beneficial traits into a homozygous potato line has now for the first time been successfully shown.

Based on the means and methods provided herein, the skilled person will be able to successfully produce homozygous potato breeding lines based on his own germplasm. In addition, the skilled person will appreciate that means and methods are provided by which he is able to, for instance, stack additional traits into the presently provided self-compatible and essentially homozygous lines in order to establish a population of lines that are enriched for agronomically desirable traits and that can serve as germplasm for commercial breeding in their own right.

EXAMPLES

Example 1. General description of the breeding procedure and pedigrees.

Many attempts were made to generate homozygous, fertile, self compatible diploid genotypes of potato without success. These attempts included the formation of doubled haploids, and the subsequent screening for fertile flowers. Several methods were employed like anther culture to regenerate haploid and eventually double haploid homozygous plants, repeated selfings to gradually increase the level of homozygosity in existing diploid germplasm and prickle pollination by using S. phureja as pollen donor to induce embryo formation from unfertilized egg cells. Especially the latter method was done at a large scale, by analyzing more than two million offspring but without the desired success. None of these tests resulted in vigourous self compatible plants.

An essentially homozygous potato line was obtained by multiple generations of selfing of plants of a line derived from public germplasm which plants have the self-compatibility controlling *Sli* gene (Phumichai et al. Euphytica (2006) 148: 227–234). These plants, designated "H" or IVP07-1001/4 herein, grew poorly, had very few flowers and each berry contained only few seeds. Moreover, the plants were very small (max 60 cm). The plants produced almost no tubers and those that formed were very small (average tuber weight about 5 grams/tuber). In all, the plants were of no value to commercial potato breeding, as they exhibited a plant weight and tuber yield that was less than 20% of that of diploid breeding clone RH89-039-16. The plants merely contained homozygous alleles on many of the loci in their genome, were self-compatible and diploid.

In the summer of 2008 plants of this IVP07-1001/4 line were crossed with different diploid donor lines (D1, D2 and D3, See for complete pedigree figure 2). D1 is early, has long sized oval tubers, light yellow flesh, and good cooking fries making quality and good cooking quality and was used as male parent (Table 6). The resulting progeny is referred to as F1(D1) in this example. Other F1 offspring plants were obtained in the similar way but using donor D2 as a female parent. D2 is an elite diploid breeding clone that is essentially non-homozygous, and is not self-compatible,

but which has the desirable agronomic trait of early cooking, oval tubers, yellow flesh and good cooking chips making quality). This resulted in lines designated as F1(D2).

The above F1's were (D1) was selfed to produce an F2(D1). This line was deposited under breeders reference AGVD1. It is estimated that AGVD1 is about 50% homozygous, meaning that about 50% of the loci for which the parents differ are homozygous.

F1(D1) was crossed with F1(D2) to produce a pseudo F2. This line (F2(D1/2)) was deposited under breeders reference AGVD2. It is estimated that AGVD2 contains about 25% homozygous alleles originating from IVP07-1001/4.

The F1's were(D1) was also backcrossed with IVP07-1001/4 to produce a BC1's line. This line (BC1(D1) and BC1(D2)) was deposited under breeders reference AGVD3. It is estimated that AGVD3 is about 75% homozygous for the loci for which the parents differ.

Other F1 offspring plants were obtained in a similar way by using donor D3 as female parent. D3 is a diploid breeding clone with yellow flesh and with the resistance genes R3, H1, Gpa2, RXadg. This resulted in a line designated F1(D3).

Similarly as described above, F1(D3) was selfed to generate an F2(D3) and backcrossed with the Sli gene donor to generate BC1(D3) offspring.

F1(D1) was crossed with F1(D2) to produce a pseudo F2. This line (F2(D1/2)) was deposited under breeders reference AGVD2. It is estimated that AGVD2 is about 25% homozygous for the loci for which the parents differ.

F1(D1) was also backcrossed with IVP07-1001/4 to produce a BC1 line. This line (BC1(D1)) was deposited under breeders reference AGVD3. It is estimated that AGVD3 is about 75% homozygous for the loci for which the parents differ.

The BC1's were selfed in a winter nursery resulting in BC1S1 populations. Next summer (2010) the F2's, the pseudo F2's and the BC1S1's were sown in seed beds in a nursery and transplanted in small peat pots. In June 2010 the seedlings were transplanted to the field at a clay and at a sandy soil in The Netherlands. In total more than 15000 plants were grown from seedlings and selected for self compatibility and for good tuber quality and yield. Most F2 and BC1S1 populations showed a very low frequency of self-compatibility. Rare plants with reasonable good berry- and seed set were selected for progeny testing without further agronomic evaluations. Only two F2 populations, originating from the D2 x H cross, showed

about 25% self compatibility. For each of the most self-compatible populations maximally fifteen plants with the best tuber quality and quantity were selected for progeny testing.

Plants of the pseudo F2's showed much more vigour than the other populations, probably as these plants do not harbour any locus with homozygous alleles of the donor parents. One of these populations showed more than 50% self-compatibility. Similarly as described above, also maximally fifteen plants of the most self-compatible populations were selected for progeny testing.

In addition, crosses were made with self-compatible plants from the field as pollen donor on two to twelve plants of the donors D1-D19 (Example 3). After evaluation of the tuber quality and quantity only crosses with selected plants or their full sibs, in case of lack of sufficient numbers of crosses, were selected for progeny testing.

In this way, in each generation, plants are selected for self-compatibility and good agronomic performance. Seeds of the selfed berries of these selected plants are collected for further testing in order to generate homozygous lines. In addition, crosses of the selected plants with other diploid donors or other breeding lines are made to enhance the genetic variation. These F1 seeds are heterozygous and several rounds of inbreeding are needed to generate homozygous offspring with the desired traits.

In this way two generations of selections, selfings and crosses can be done per year. This process is even further enhanced by exploiting marker aided breeding by using diagnostic markers for specific traits and random markers to select for the recurrent parent genome. When elite parents have been obtained, derivatives of these lines can be made by two to three generations of backcrossing and two generations of selfing.

Example 2 Breeding self compatible diploid potato.

1. Introduction

A common characteristic of diploid potato is self-incompatibility (SI); upon self-fertilisation no vital progeny is generated. The S-locus inhibitor gene (*Sli*)

originating from *Solanum chacoense* has been described (*Euphytica* 99,191-197, 1998) to inhibit this self-incompatibility and rendering diploid potato self-compatible (SC). Upon repeated selfings, close to homozygous self-compatible diploid potato clones have been generated (Genome 48, 977-984, 2005). The agricultural quality of these clones was very poor indeed reflecting a poor germination, plant growth, tuber quality and tuber yield.

2. Experiments

2.1 Materials

In order to develop self-compatible potato clones with much higher level of agricultural traits we crossed the *Sli* gene donor with various potato clones, designated: IVP97-079-9, IVPAA-096-18 and SH83-92-488. These clones are available at Dr. R.C.B. Hutten, Laboratory of Plant Breeding, Wageningen University, The Netherlands. As used herein, these clones are designated as clones D1, D2 and D3 resp. The progenies (F1) were selfed and backcrossed to the *Sli* gene donor generating F2 and BC1 populations, respectively. The BC1 was selfed to generate BC1S1 populations. All these crosses and self fertilisations were performed without any other selection than self compatibility of the plants.

2.2 Evaluation of progenies in a wintertrial

In a pilot experiment several random BC1 and F2 progenies were grown in a heated greenhouse with 16 hours artificial light in while outside temperatures were below zero for almost three months. We evaluated the self-compatible % as the number of plants with berries after hand pollination and the yield as the weight of tubers per plant (Table 2).

Results

The *Sli* donor is self-compatible (SC) and most of the F1 and BC1 plants were also self-compatible, while a minority of the F2 plants was self-compatible. The *Sli* donor showed an extremely low tuber yield, while the BC1 and one F2 was about 50% of the F1 tuber yield. The tuber yield of some BC1 plants was close to that of the F1 plants and some (unselected) F2 plants were better than the weakest F1 plants.

Table 2. Some characteristics of winter grown plants of a diploid potato breeding program to introduce the *Sli* gene.

	# plants	compatibility	yield (gram per SC plant)	
		frequency (%)	average	variation
Sli donor	2	100	22*	0 - 43
F1 (HxD3)	9	89	443**	258 - 634
BC1 (Hx(HxD1)	49	81	206**	0 - 524
BC1 (Hx(HxD2)	64	72	237**	28 - 543
F2 selfed (HxD1)	23	22	252**	131 - 376
F2 selfed (HxD2)	20	25	69**	0 - 201

* from tuber

** from seedling

2.3 Evaluations of progenies in the field

In the summer of 2010 progeny plants of F2 and BC1S1 populations were tested in the field to select self-compatible progeny plants with good agricultural performance (Table 3).

Table 3. Breeding scheme to introduce self-compatibility (SC) into diploid potato. In bold the most promising materials are indicated.

P	F1 (HxD)	F2: selfed (HxD)				BC1S1: selfed (Hx(HxD))			
parents	plants (DxH)	Populations tested	plants in the field	self compatible plants	plants selected*	populations tested	plants in the field	self compatible plants	plants selected
	#	#	#	#	#	#	#	#	#
H = *Sli* donor									
D1	33	15	2460	± 50	7	38	2521	< 10	1
D2	45	29	***5598***	***> 500***	***52***	24	1380	< 10	1
D3	8	8	1602	± 10	7				

* sufficient seedset

Results

We tested 52 F2 populations comprising 30 to 840 plants and 62 BC1S1 populations comprising 1 to 390 plants, in total 9660 F2 plants and 3901 BC1S1 plants resp. Most of the populations showed a weak plant growth and a low frequency of self-compatibility. Only two F2 populations derived from the D2 parent showed about 25% self compatible plants. The best plants of these populations were selected on the basis of tuber yield and tuber quality.

3. Conclusion

Only two out of the 52 F2 populations and none of the BC1S1 populations showed a reasonable level of self-compatibility. However, unexpectedly, the desired plants could be obtained, starting from material that would otherwise have been neglected by breeders. The main differences of the plants of the invention and those of the prior art are given in Table 4 below.

Table 4. Characteristics of the parental potato lines of the present invention proposed for the production of hybrid (true) potato seed.

	Vigour	Ploidy	Tuber yield	Fertility	Self-compatibility.
Sli-gene donor (Phumichai et al. Euphytica (2006) 148: 227–234).	low	2n	low	low/ intermediate	high
Diploid trait Donor (e.g. D1)	intermediate	2n	intermediate	intermediate	low
Commercial variety	high	4n	high	high	low
Lines of present invention	Intermediate /high	2n	Intermediate /high	low/ intermediate	high

Example 3 Development of a molecular marker platform

Introduction

Numerous marker systems are available to support genetic research. For the present invention a molecular marker platform in potato was developed and used to evaluate the level of homozygosity versus heterozygosity in diploid potato.

Selection of most informative sequences for SNP assays

In order to analyse SNPs in potato, the Illumina standard GoldenGate protocol (Shen et al. 2005. Mutation Research 573:70–82) has been used previously (Anithakumari et al. 2010. Mol. Breeding, 26:65-75). This platform comprises 384 SNPs and was later expanded with another 768 SNPs. These 384 and 768 SNPs have been developed using EST sequences, which represent the mRNA without intron sequences. Since the present inventors have access to the first draft sequence of the potato genome (www.potatogenome.net) it was possible to compare the mRNA with the genomic DNA sequence including the intervening sequences. This comparison with the potato genome sequence was performed with the Basic Local Alignment Search Tool (BLAST; Altschul et al. 1990. J. Mol. Biol. 215: 403-410). This resulted in 538 and 1681 hits, respectively. The occurrence of multiple BLAST hits was considered to reflect paralogs or intron spanning sequences. These were removed from

the initial collection of SNPs that were first developed for GoldenGate analysis (Anithakumari et al. 2010). This resulted in 279 and 453 unique BLAST hits representing the occurrence of only one copy in the potato genome sequence of *Solanum tuberosum* Group Phureja DM1-3 516R44 (CIP801092) as known hitherto (Potato Genome Sequencing Consortium, www.potatogenome.net).

From the total of 732 SNPs with unique BLAST hits only a few (31 cases, equally distributed over the 384 and 768 set) were omitted due to high level of sequence mismatches (3-6 SNPs within 101 bp). One mismatch should be expected and a second SNP was tolerated to be included in the SNP-spanning sequences selected.

For the present SNP design the minimal sequence length was set at 101 bp. Therefore 81 sequences were removed as they were shorter. The resulting 553 sequences have a length of at least 101 bp. A total of 100 sequences were then removed for having incomplete matches of only 35 up to 94 nucleotides within a scaffold of a doubled monoploid (DM-3 516R44; www.potatogenome.net). Also removed were sequences with introduced alignment gaps and sequences having other potential mismatch problems.

Though twenty-five sequences did not meet the criteria described above, they were kept as these were were known to be highly informative in the GoldenGate SNP assays. These SNPs even allowed to discriminate between the five genotype classes that occur in tetraploids (Voorrips et al, EAPR-EUCARPIA congress "Potato Breeding after completion of the DNA Sequence of the Potato Genome", 27-30 June 2010, Wageningen, the Netherlands, e-publication at http://edepot.wur.nl/143559, pg 42).

Finally, 515 sequences were selected. The final selection of 100 sequences was based on the known map position of 237 markers (out of the 515) on the potato genetic map (Anithakumari et al, 2010 and Van Os et al. Genetics 173: 1075-1087, 2006). The selected markers were evenly distributed over the 12 potato chromosomes including the most distal telomeric as well as centromeric markers and with a preference for SNPs that were heterozygous in clone RH (RH89-039-16; www.potatogenome.net), because clone RH is a progenitor of the material used in the present research. Or in other words, the alleles of clone RH are also present in material used in the present research (see Table 2 and Figure 3).

Testing of markers

The 100 SNP sequences selected as described above were used in a KASPar SNP genotyping system (KBioscience Ltd. Hoddesdon, UK; http://www.kbioscience.co.uk/lab%20services/SNP%20Genotyping/genotyping_chemistry.html). The markers were tested on a set of 20 diploid potato genotypes (see Figure 4) including the three diploid parents used in the present study and further on the donor of the *Sli* gene, clone 07-1004-1 (see also Example 2 herein above).

Results

All SNP sequences generated polymorphisms in the KASPar SNP genotyping system (see Figure 4), corresponding to a 100% success rate. In total, 100 markers x 20 genotypes were tested, comprising 2000 datapoints. We observed 29 missing data, which is less than 2 %. As control, one genotype was tested twice whereby plant samples from different locations were used (Figure 4, columns 4 and 7). All data were identical. These results illustrate that the KASPar SNP genotyping system is an efficient, reliable and reproducible marker platform for diploid potato. This indicated that The KASPar SNP genotyping system is efficient, reliable and reproducible for diploid potato.

All markers showed a high frequency of occurrence in the diploid potato germplasm used in this experiment (Appendix III). The heterozygote frequency in the germplasm by suing these 100 markers ranged from 26 % to 77%, while the *Sli* gene donor was 100% homozygous as is in accordance with expectations (Phumichai et al. 2005 Genome 48: 977–984).

The number of informative markers between the Sli donor and the three parents used in the present study was 54, 52 and 62 resp. This indicates that the KASPar SNP genotyping system is also informative for diploid potato.

Based on the above, it was concluded that the KASPar SNP genotyping system as described herein and based on the markers as developed herein is efficient, reliable and reproducible and very informative to describe the level of homozygosity / heterezygosity in diploid potato.

Table 5A. Sequences of 100 SNP markers in potato

SNPs				
SNPID	SNPNum	AlleleY	AlleleX	Sequence
PotSNP002	11940065	G	C	ACCTGGTGCAGGCCT[C/G]CGTATTGATACGATT
PotSNP011	11940098	C	T	GACTGTGATTCGAAA[T/C]GTTTACCTATCTCTT
PotSNP021	11940075	C	T	TCGTAGAAATGACAT[T/C]TGGGGTGCTAGAGAT
PotSNP026	11940044	C	T	AGTTTCCAGCTGATG[T/C]TATTCTAGGAGACGA
PotSNP032	11940037	G	A	AGAGGCTCTTGAGGC[A/G]CAGCGAAAAGAGGAA
PotSNp038	11940016	C	T	TGGTGACGTTCATGT[T/C]GGACGTAATGCTTCT
PotSNP043	11940036	C	A	AGTATCTCCGTCCGA[A/C]TCACAGTCCTACTCT
PotSNP045	11940094	G	A	GGTGCTAAGATTCGC[A/G]TTGATGATTCACTAG
PotSNP047	11940054	C	T	CCAACACTGGAAAAA[T/C]TATGGAAGCAGAAAG
PotSNP052	11940097	C	T	GCTAGCATTAGAGCT[T/C]GCTAAGCGACTCAAT
PotSNP055	11940021	G	A	GCCTTGGAGTAAGAG[A/G]TTTTCTGTTTTGGGC
PotSNP057	11940040	C	T	GGATGTTCATCAACG[T/C]CGACTTCGACAAGCT
PotSNP061	11940032	C	T	ACAACCTGCAAGAGC[T/C]TTGCATCTGTTTAGT
PotSNP068	11940023	C	T	TACAATCATGATCCT[T/C]TTGCCTAATTGCCCT
PotSNP072	11940005	G	A	CTGAGCTAGTAGATC[A/G]ATCAAACGGTGCTCC
PotSNP073	11940076	G	T	GACTGTATGGACAGT[T/G]ATTGGGTCAGAGGAC
PotSNP080	11940068	C	T	AAGATGACGTGGAGT[T/C]CGGCACTGCGCCGTT
PotSNP081	11940047	C	T	GCCGCACAAGGTGCT[T/C]GCCGTCGATGTTAGC
PotSNP083	11940033	C	T	AGGTAGTGTTGCAAA[T/C]ACAATTAGAGGGTTG
PotSNP089	11940062	G	A	AAGTACATTTATGAG[A/G]AGATTAACAAGTGTT
PotSNP099	11940092	C	T	ACTGCTGGAACTGAG[T/C]TGAACAATGCTATTA
PotSNP100	11940083	C	T	GAGTGCCGGCAGCGT[T/C]ACAGATCCGACGATG
PotSNP102	11940070	G	A	TATGGCAGAGAAAGA[A/G]ATGGAGTATAGAGTG
PotSNP1026	11940059	G	C	TAAACTCCAACATAT[C/G]AACACAAATTAGTGC
PotSNP1034	11940001	G	A	TTCTACATCCTTCGC[A/G]GGCATTTTTGAGGAG
PotSNP1049	11940051	T	C	TTTGGGGAGGAACAC[C/T]TGTGCTTTAGAACTC
PotSNP1057	11940022	A	C	TGTCACTAAGAAAGC[C/A]CAAGAGGCAATGCAG
PotSNP106	11940010	G	T	GCAAGCTACTTGTTC[T/G]AAAGGACCTGCTACT
PotSNP1072	11940027	G	T	AGCGAGTTTGGATGC[T/G]GAGCTTTTGCAGTTA
PotSNP1083	11940019	C	T	TACAAAGTTCACTGC[T/C]GAGGAGGTCATGCAG
PotSNP1105	11940041	A	C	GATGAGGAAAGGCAC[C/A]GGCGGAGGTGTTGGA
PotSNP1115	11940071	T	C	CACCACCGTCCAAAC[C/T]GGTTTTGCCAATCGC
PotSNP1122	11940060	T	C	GGTCCCTGAGCAAGG[C/T]AATCAACGAATTAGG
PotSNP1139	11940004	G	A	AGTCTCTACAAACCC[A/G]TGGTTTCGATCTTGA
PotSNP116	11940058	G	A	TAATGTGGTCGGCAC[A/G]CTGACGCTTGCTGAT
PotSNP118	11940091	G	A	AGATGCATTCTATGC[A/G]GAACCACAAAGATAT
PotSNP120	11940082	G	A	TTCGTGTTGCACTCA[A/G]CGATTCAGTTGGATG
PotSNP121	11940099	C	T	AGCTTGTGCTAATAG[T/C]TGTGCTGATTTTCTC
PotSNP122	11940078	G	A	TATGCAATCCCAGCC[A/G]ACATATCCGATCCAT
PotSNP124	11940055	G	A	TCATACTTCACATAT[A/G]TCAGAGTGGTCTGAT

PotSNP130	11940050	C	T	AGAGAAAAAAGCTGT[T/C]GTTGTTGATGACGAG
PotSNP134	11940080	C	T	CGCGTATTTCCCTTA[T/C]TATAACTCGTTCTCT
PotSNP138	11940052	C	T	TTACATATGCAATAG[T/C]GGTGGTGATCTTTAT
PotSNP152	11940077	C	T	GAAGTTGTCTTACAT[T/C]GCTCTTGACTATGAA
PotSNP162	11940074	C	T	CTATGATGCATTTAG[T/C]TCATGGAGCAAACTT
PotSNP165	11940009	G	A	AGTTCCTTTTTCTCC[A/G]AGTTCTCAGACTCCG
PotSNP178	11940026	G	T	GAGGAATTGATGCGT[T/G]CTGAACTGGCACAGT
PotSNP182	11940095	G	A	ATACACAGGAAGGGT[A/G]AAGTGCTTCAAATTG
PotSNP184	11940038	G	A	TTTGACCATTCTTTC[A/G]GTCCTAGCTAACCAC
PotSNP185	11940002	C	T	AAGATAAGAAGAAAG[T/C]TAAGAGAACCAAGAT
PotSNP194	11940064	C	T	GGCCCACTTTCCACA[T/C]ATTCAATTTGGTCTC
PotSNP205	11940042	G	A	TCAACTCACCACTGG[A/G]TTTTACTCAAAATCA
PotSNP213	11940096	G	A	TAATATGTTGTGTCT[A/G]TAATTTTTGGGTCAT
PotSNP220	11940013	C	T	CATTAACCAAGGTTG[T/C]ATCGTTCGAGAGATC
PotSNP229	11940056	C	T	TCCTTTGTCATTGTA[T/C]GCCATGGAAGAAGCA
PotSNP392	11940003	T	A	GCTTTGTTTAAGAAC[A/T]GCCAGAAGAAGTTGC
PotSNP401	11940012	G	A	GCCTACGCGGCATGA[A/G]GATGTGGATATTGTT
PotSNP402	11940072	T	C	AAAGCTCAAATTAAA[C/T]GACGATGTTCAGGAA
PotSNP423	11940020	A	G	GGCCAACCAATTCCA[G/A]CGTCTTCTCCCAGAC
PotSNP446	11940081	C	T	AAAACCATTTGGTCT[T/C]GTTGATAATTCTGAG
PotSNP458	11940069	A	G	TACATAGCTCTACAA[G/A]CTTGGAAACATGTCA
PotSNP470	11940043	T	A	GACACAAAATTCATA[A/T]TGGCTGATACTTTGT
PotSNP543	11940049	T	A	GCAACTTGTCGTCGG[A/T]AAACTCGACTAGATT
PotSNP567	11940014	A	C	CTAGAAACAAAATCA[C/A]AATATAAGTTACATA
PotSNP569	11940028	G	A	GTTGTTTGCTTTGTT[A/G]TGCTGACTTATTGTA
PotSNP573	11940034	C	T	ATGCTTCTTGGTTGC[T/C]GTTTGTTTAGGCTCT
PotSNP580	11940087	T	C	AAATATGTTGTTCCA[C/T]TAGAAAAAAGAGTAA
PotSNP586	11940088	C	T	GTGAATACTGGTATC[T/C]TTCCAATTGAAGTCA
PotSNP587	11940067	C	T	AATATTCTTGTAGTA[T/C]ATATTGTCTAAAATC
PotSNP607	11940084	T	C	CAACGATATCGAGAA[C/T]TATTGTTCTAAGGGA
PotSNP626	11940045	G	A	GCTGAAAGTAAAGAC[A/G]GTGTTCAACATCTTG
PotSNP645	11940007	T	G	CATGCAATTGTAATA[G/T]TATCAATGTTTGTTG
PotSNP652	11940024	T	C	TGCTCAAGCTGCCAA[C/T]GCTAATCCGCTTTAC
PotSNP700	11940061	G	A	CAAGATATATGCAGC[A/G]GAGTCCCTTCGAGCA
PotSNP702	11940039	A	T	TTATGGTTATGGTGG[T/A]GGAGGTTGGATTAAT
PotSNP712	11940057	C	G	GGGAGGGTGAAAATA[G/C]TCGAAAACGGGCAAC
PotSNP713	11940073	G	A	GATTGTTATCCTTCC[A/G]CCACCTCCAACAGAT
PotSNP731	11940035	A	G	CAACTTCCAGTCGTG[G/A]GAGTGGGGTTAACGG
PotSNP753	11940046	G	A	ATGGTGTCTCCAAGG[A/G]ACTATTACAGTCACT
PotSNP759	11940090	C	T	AACATCGATAGGACT[T/C]TGAAGGCCGCGGATG
PotSNP766	11940089	T	C	CTGCCACTGCGGCTG[C/T]AGGTTCTGCAGCTGC
PotSNP775	11940079	G	A	TGAAATGATTGGATT[A/G]TGACCCATAGTTTAG
PotSNP796	11940025	C	A	ATCTCTAAGTTGGGG[A/C]AACCCATAATTTCTA
PotSNP827	11940053	T	A	CGCTTGGGAAGAACC[A/T]TTCGGTCCAGTTTTG

PotSNP834	11940006	T	C	CCAATTCCGACGGCC[C/T]TCGTCGTTTCACCGC
PotSNP853	11940066	A	C	GGTCGACCATTGGGG[C/A]CCCGTAGAGGGTATA
PotSNP866	11940048	G	T	AAGGCGAATTCCGGT[T/G]CCCGAAATGGATCTC
PotSNP893	11940011	A	G	TCATTACATCAAAAT[G/A]TTTTATCAATGCCCA
PotSNP908	11940100	T	A	TGATCCATTTGGTCT[A/T]GACCAGTTCTTGACA
PotSNP910	11940017	T	A	TGCTGTGCTCCTTCT[A/T]TTCCTCATCTCTCAC
PotSNP928	11940030	A	T	TCATTCTCACTCTCC[T/A]GCTGAACAAGCCATT
PotSNP947	11940015	T	G	AAAAGACAAGAATCT[G/T]GTTCAGATTCTCCAG
PotSNP948	11940063	T	A	TGGGGAAAACTTAAT[A/T]TGACAGCCAAGGCGT
PotSNP960	11940031	C	T	GGTTTTGTGGACTGC[T/C]AACACTGAAAGATAC
PotSNP964	11940029	A	T	TTATTTCAATGAAGC[T/A]TCTGGTGGACGTTAT
PotSNP982	11940085	C	T	TCTGTGGTTTTTTCA[T/C]CACCTCCATCTTCAA
PotSNP983	11940086	G	A	TAAGATTAGTTCTAA[A/G]CTTAGCGCAGAAGAC
PotSNP985	11940018	A	G	GAGCTCTATAAGTGC[G/A]CCTGTTACTCCACCT
PotSNP987	11940008	C	A	GGGCTATGGTTGGTT[A/C]GGAAAAAAGGTGCAA
PotSNP996	11940093	C	T	GGATATTGGGTTCGT[T/C]CCATGATTCGTGGCA

Table 5B: List of PotSNPmarkers and their specific annotation for position on the SH and RH map of Figure 3.

Marker	Annotation	Marker	Annotation	Marker	Annotation
PotSNP072	1.1	PotSNP573	5.1	PotSNP102	9.1
PotSNP834	1.2	PotSNP184	5.2	PotSNP587	9.2
PotSNP645	1.3	PotSNP702	5.3	PotSNP1115	9.3
PotSNP1034	1.4	PotSNP731	5.4	PotSNP402	9.4
PotSNP987	1.5	PotSNP043	5.5	PotSNP713	9.5
PotSNP185	1.6	PotSNP057	5.6	PotSNP080	9.6
PotSNP392	1.7	PotSNP1105	5.7	PotSNP458	9.7
PotSNP165	1.8	PotSNP032	5.8	PotSNP162	9.8
PotSNP1139	1.9	PotSNP205	6.1	PotSNP021	10.1
PotSNP038	2.1	PotSNP753	6.2	PotSNP073	10.2
PotSNP910	2.2	PotSNP470	6.3	PotSNP152	10.3
PotSNP106	2.3	PotSNP026	6.4	PotSNP122	10.4
PotSNP893	2.4	PotSNP626	6.5	PotSNP775	10.5
PotSNP401	2.5	PotSNP081	6.6	PotSNP134	10.6
PotSNP220	2.6	PotSNP866	6.7	PotSNP446	10.7
PotSNP567	2.7	PotSNP124	7.1	PotSNP120	10.8
PotSNP947	2.8	PotSNP047	7.2	PotSNP586	11.1
PotSNP055	3.1	PotSNP543	7.3	PotSNP100	11.2
PotSNP985	3.2	PotSNP130	7.4	PotSNP607	11.3
PotSNP1083	3.3	PotSNP1049	7.5	PotSNP766	11.4
PotSNP423	3.4	PotSNP229	7.6	PotSNP759	11.5
PotSNP1057	3.5	PotSNP712	7.7	PotSNP118	11.6
PotSNP068	3.6	PotSNP138	7.8	PotSNP982	11.7
PotSNP652	3.7	PotSNP827	7.9	PotSNP983	11.8
PotSNP796	3.8	PotSNP1026	8.1	PotSNP580	11.9
PotSNP178	4.1	PotSNP1122	8.2	PotSNP099	12.1
PotSNP1072	4.2	PotSNP700	8.3	PotSNP011	12.2
PotSNP960	4.3	PotSNP853	8.4	PotSNP996	12.3
PotSNP061	4.4	PotSNP089	8.5	PotSNP045	12.4
PotSNP569	4.5	PotSNP948	8.6	PotSNP121	12.5
PotSNP083	4.6	PotSNP194	8.7	PotSNP908	12.6
PotSNP964	4.7	PotSNP116	8.8	PotSNP182	12.7
PotSNP928	4.8	PotSNP002	8.9	PotSNP213	12.8
				PotSNP052	12.9

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Table 6. Diploid potato genotypes used for SNP marker development and testing

Nr.	Abbr.	code	Short description	reference
1	H	07-1004-1	*Sli* gene donor	07-1004-1 is a progeny of the *Sli*-donor F1-1 as described in Phumichai et al. (2005) Genome Vol. 48:977-984, Phumichai and Hosaka (2006) Euphytica 149:251–258 and Phumichai et al. (2006) Euphytica 148: 227–234.
2	D1	IVP97-079-9	Parent 1 used in present research, early, long, Y (yellow flesh), Qcook	Examples 1, 2 and 4 herein; available from Ronald Hutten, Laboratory for Plant Breeding, Wageningen University.
3	D2	IVPAA-096-18	Parent 2 used in present research, early, Y, Qcook	Examples 1, 2 and 4 herein; available from Ronald Hutten, Laboratory for Plant Breeding, Wageningen University.
4	D3	SH83-92-488	Parent 3 used in present research, *R3*, *H1*, *Gpa2*, *RXadg*, Y	Examples 2 and 4 herein; available from Ronald Hutten, Laboratory for Plant Breeding, Wageningen University.
5	D4	parent C	Parent of the CxE population	Anithakumari A.M. et al. (2010) Molecular Breeding 26(1):65-75
6	D5	parent E	Parent of the CxE population	Anithakumari A.M. et al. (2010) Molecular Breeding 26(1):65-75
7	D3	SH83-92-488	Independent duplo of nr 3	Van Os, H. et al. (2006) Genetics 173(2):1075-1087
8	D6	RH89-039-16	Used for genome sequencing	Van Os, H. et al. (2006) Genetics 173(2):1075-1087; available from Ronald Hutten, Laboratory for Plant Breeding, Wageningen University
9	D7	3778-16	*Grp1*, early, long (shape), *Ro1*	Rouppe van der Voort et al. (1997) Mol Gen Genetics 255(4):438-447
10	D8	IVP92-057-3	early, long, Y, Qfry	Used for marker development in Example 3
11	D9	IVP98-082-14	Long, Y, Qfry, *H1*, Qcook	Used for marker development in Example 3
12	D10	IVP02-089-5	Early, long, Y, *H1*, Qcook, Zep (orange flesh)	Used for marker development in Example 3
13	D11	IVP05-113-1	Early, y(white flesh)	Used for marker development in Example 3
14	D12	IVP05-122-24	Qstarch, Y	Used for marker development in Example 3
15	D13	IVP06-142-12	Wild species	Used for marker development in

			hybrid: Phyt avl	Example 3
16	D14	IVP06-145-2	Wild species hybrid: Phyt rch	Used for marker development in Example 3
17	D15	IVP06-148-13	Round (shape), Qcook, Qfry	Used for marker development in Example 3
18	D16	IVP06-149-12	Early, round, Zep, Y, spectacled, Qcook, bluc anthocyans	Used for marker development in Example 3
19	D17	IVP06-155-9	Wild species hybrid: Phyt tar	Used for marker development in Example 3
20	D18	IVP06-161-16	Early, Phyt vnt1, round, Y, *H1*	Used for marker development in Example 3
21	D19	IVPAA-134-16	Early, round, y (white flesh)	Used for marker development in Example 3

Early: early flowering (short cycle); Qcook: good cooking quality; Qfry: good frying quality; Phyt indicates *Phytophthera* resistance genes

Example 4. Development of homozygous diploid potato plants and generation of heterozygous F1 hybrid progeny

Introduction

The objectives of this Example were to develop homozygous diploid potato lines that are self-compatible (SC) and have good agronomic performance. A breeding programme to combine self-compatibility with good agronomic performance is described above in Example 2 above. The aim of the current experiment is to prove that homozygous diploid self-compatible potato genotypes can be generated by inbreeding and that diploid potato plants containing contrasting homozygous loci scattered over the potato genome can be crossed (are intercrossable) to generate heterozygous F1 hybrid progeny.

Materials and methods

A self-compatible diploid homozygous potato genotype, that harbours the *Sli*-gene as described in the Examples above was crossed with two potato clones, designated D1 and D2 (see Examples 1 and 2) The F1 progenies were selfed and two F2 progenies were generated from two random SC F1 plants. Random self-compatible plants of these two F2 populations were selfed and per F2 population three F3 populations were tested for level of homozygosity and for segregation of SNP markers.

The 6 F2 plants were tested for ploidy level (by fluorescent quantitation of DNA) and were all found to be diploid.

A set of 36 markers was used on 265 individual F3 plants by applying the KASPar SNP genotyping system (see Example 3) covering all 24 chromosome arms of potato (see Figure 5).

Results

Subsets of 35 to 56 plants from the six F3 populations, in total 265 plants, were tested with at least 24 informative markers per parent pair. In total 36 markers were used (see Figure 5). For position of markers on the potato genome see Example 3, above. The *Sli* donor was 100% homozygous for these markers tested, while the D1 and D2 parents contained 20 and 19 loci resp. that were heterozygous out of the 24 informative markers tested per parent pair, while the other five and six loci resp. contained homozygous alleles originating from the from the donor parent (D1 or D2).

Six homozygous F3 plants

As expected, the F3 populations segregated for markers that were heterozygous in the F2 and not for markers that were fixed in the F2 (Figure 5). The mean heterozygote level ranged from 84 to 95 %, while that of individual plants ranged from 71 to 100% (Table 7).

Table 7. Homozygote frequencies (%) of six F3 populations

F3 population	Donor parent	mean	range
126	D1	85	74 – 96
126B	D1	88	74 – 100
127	D1	84	71 – 96
121	D2	85	71 - 96
122	D2	89	79 -100
123	D2	94	88 - 100

Three F3 populations of the D1 x H and of the H x D2 crosses were analysed for frequency of homozygous loci based on the data in Figure 5 as shown in Table 9.

These frequencies are expected giving the allele frequencies of the parents. There were six plants from three populations that were already 100 % homozygous.

These harboured mainly homozygous loci with *Sli* donor alleles. This is expected as the *Sli* donor harbours homozygous alleles, while the other parents contained one common allele with the *Sli* donor per heterozygous marker. So, *Sli*–donor derived alleles were abundant in all offspring populations. These six homozygous F3 plants, designated 122-34, 123-6, 123-20, 123-23, 123-24 and 126B-17, contained two, five, four, three, five and seven loci resp. with homozygous alleles from the donor parent. So, already in two selfed generations 100 % homozygosity was reached for 24 marker loci representing 24 chromosome arms.

No absolute lethal allele

It is often stated that repeated selfings in diploid potato will only result in weak plants due to inbreeding depression. This should be due to numerous deleterious alleles that reduce plant fitness, when these alleles are homozygously present. Therefore, we tested the segregation of 36 markers in the six F3 populations mentioned (see Figure 5).

These markers, that were chosen because they are informative for the parent pairs used in this study, were also tested for the frequencies in 19 diploid potato genotypes, that were chosen as donors for the breeding programme (see Example 3, Figure 4 and Table 9). If lethal alleles are present, then loci with homozygous alleles should be absent. Indeed, seven markers (designated PotSNP165, PotSNP205, PotSNP753, PotSNP 238, PotSNP 700, PotSNP 100 and PotSNP 607) did not show homozygous alleles that were different from the *Sli*-donor alleles in this diploid germplasm. Most of these markers were already fixed in the F2, most of them for *Sli* donor alleles as these were more abundant in the parents, but also examples were observed whereby the F3 population was fixed for the allele of the non-*Sli* parent (PotSNP753 in populations 126B and 127). Three of these seven markers (PotSNP205, PotSNP753 and PotSNP100) segregated in the F3 populations, whereby the PotSNP753 segregated normally and PotSNP205 and PotSNP100 showed distorted segregation. These may represent deleterious alleles. Only one plant with homozygous PotSNP100 donor alleles was identified. Without wishing to be bound by theory, it is believed that this may represent a very deleterious allele. As a single plant has been identified with a homozygous allele of the donor parent, this does not provide full proof for an absolute lethal allele.

F3 x F3 crosses

The 265 F3 plants tested were raised in an unheated greenhouse in the summer of 2010. In addition about 600 additional F3 plants were raised in the field with clay soil (see also Example 2). All these plants were from unselected F2 plants grown in a winter-nursery in The Netherlands (see Example 2). Crosses were made by applying pollen from greenhouse and field grown self-compatible plants on stigma of greenhouse grown self-compatible F3 plants. Only crosses were made between F3 plants with different great-grandparents (F3 plants of populations 121 - 123 crossed with 126 – 127). The greenhouse plants designated 126-24, 126-31, 126B-2 and 127-21 were successfully crossed as female self-compatible parent with a self-compatible F3 plant from population 123 as male parent. The female F3 plants were more than 80% homozygous, while the F3 plant in the field was not tested but the population mean homozygosity level was 94% with a range from 88 to 100%.

The parents of the F3 x F3 crosses were compared for loci that are already fixed in the F3 populations (see Table 8). Ten homozygous loci on nine chromosomes were identified that were at least contrasting between one of the four female and the one male parent used for the F3 x F3 crosses. As a consequence, the F1 hybrid progeny will be at least heterozygous for those contrasting loci. As each individual parent pair contained at least five contrasting loci, each individual progeny plant will harbour at least five contrasting chromosome regions.

Table 8. Contrasting fixed loci between the parents of the F3 x F3 crosses.

		chr	126-24	126-31	126B-2	127-21
Homozygosity level	%		86	80	88	88
Contrasting homozygous loci with F3 population 123	PotSNP392	1	x			
	PotSNP038	2	x	x		x
	PotSNP985	3			x	x
	PotSNP1105	5	x	x	x	x
	PotSNP753	6			x	
	PotSNP124	7				x
	PotSNP162	9	x	x	x	
	PotSNP021	10			x	x
	PotSNP120	10	x	x	x	x
	PotSNP213	12	x	x		
Total #	10	9	6	5	6	6

The codes 126-24, 126-31, 126B-2 and 127-21 refer to individual F3 plants that were used as females in crosses with one field grown plant of the F3 population designated "123" (see above). The homozygosity level was calculated as the percentage of homozygous loci over all loci tested (Figure 5). The "x" refers to homozygous markers that with contrasting alleles between the four female plants and the one plant of population 123.

Conclusions

It was found that after two generations of selfings homozygous diploid potato plants were obtained comprising alleles from both parents. Not a single marker out of 36 markers tested provided proof for a lethal allele. Furthermore, plants with more than 84% homozygous loci can be successfully crossed to obtain hybrid plants with at least five heterozygous chromosome regions. This means that hybrid seeds that are essentially uniform and that represent true potato seed have now been produced.

Table 9. Allele frequencies of genome wide SNP markers in diploid germplasm and F3 populations.

SNP marker	chrom	allele frequencies											
code	no	diploid germplasm				F3 from H X D1				F3 from H x D2			
		HH**	HD	DD	HD	HH	HD	DD	HD	HH	HD	DD	HD
		#*	#	#	%***	#	#	#	%	#	#	#	%
PotSNP834	1	4	9	5	50	47	79	17	55	122	0	0	0
PotSNP392	1	5	11	3	58	9	32	14	58	122	0	0	0
PotSNP165	*1*	*13*	*5*	*0*	*28*	*142*	*0*	*0*	*0*	n.i.			
PotSNP038	2	2	12	5	63	15	48	80	34	122	0	0	0
PotSNP947	2	8	9	1	50	118	13	12	9	122	0	0	0
PotSNP985	3	3	8	6	47	62	17	63	12	18	46	58	38
PotSNP796	3	2	12	5	63	142	0	0	0	122	0	0	0
PotSNP960	4	6	10	3	53	59	61	20	44	n.i.			
PotSNP569	4	13	2	4	11	n.i.				121	0	0	0
PotSNP928	4	5	13	1	68	141	0	0	0	88	19	15	16
PotSNP184	5	2	7	10	37	61	59	23	41	122	0	0	0
PotSNP1105	5	2	12	5	63	140	0	0	0	19	40	62	33
PotSNP205	*6*	*13*	*6*	*0*	*32*	n.i.				*91*	*27*	*3*	*22*
PotSNP753	*6*	*9*	*10*	*0*	*53*	*28*	*20*	*91*	*14*	n.i.			
PotSNP626	6	9	8	2	42	n.i.				122	0	0	0
PotSNP081	6	12	6	1	32	142	0	0	0	n.i.			
PotSNP124	7	8	9	2	47	85	0	55	0	n.i.			
PotSNP229	7	7	11	1	58	56	0	0	0	15	37	69	31
PotSNP138	*7*	*9*	*10*	*0*	*53*	*55*	*0*	*0*	*0*	*121*	*0*	*0*	*0*
PotSNP1026	8	10	6	3	32	n.i.				80	0	42	0
PotSNP700	*8*	*9*	*9*	*0*	*50*	*140*	*0*	*0*	*0*	*34*	*0*	*0*	*0*
PotSNP116	8	7	9	3	47	140	0	0	0	n.i.			
PotSNP102	9	3	12	2	71	141	0	0	0	122	0	0	0
PotSNP458	9	11	7	1	37	n.i.				122	0	0	0
PotSNP162	9	14	4	1	21	11	31	98	22	n.i.			
PotSNP021	10	0	10	9	53	33	55	53	39	35	0	0	0
PotSNP152	10	0	8	11	42	141	0	0	0	82	23	17	19
PotSNP120	10	9	9	1	47	n.i.				48	23	51	19
PotSNP100	*11*	*14*	*5*	*0*	*26*	*53*	*89*	*1*	*62*	n.i.			
PotSNP607	*11*	*14*	*5*	*0*	*26*	n.i.				*122*	*0*	*0*	*0*
PotSNP580	11	2	5	12	26	142	0	0	0	43	53	24	44
PotSNP099	12	1	10	8	53	56	0	0	0	120	0	0	0
PotSNP996	12	7	8	4	42	142	0	0	0	n.i.			
PotSNP182	12	6	10	3	53	n.i.				56	43	21	36
PotSNP213	12	9	8	1	44	22	38	80	27	n.i.			
PotSNP052	12	4	8	7	42	142	0	0	0	89	18	14	15

#: number of plants; HH: homozygous *Sli*-donor locus; DD: homozygous donor locus; HD: heterozygous locus; % is percentage heterozygous plants, low numbers indicate high level of homozygosity in F3.

Example 5. Breeding scheme for a commercial potato variety

Starting from F3 materials obtained from crosses between an *Sli* donor and another diploid potato as described in Examples 2 and 4 above, a breeding scheme for producing commercial potato varieties is set out herein below. This scheme consists of a number of consecutive steps, which, in combination with the teaching as described herein, will allow the skilled person to arrive at a diploid or tetraploid hybrid potato variety that is as good as or preferable better then existing commercial hybrids and that can be multiplied much faster than conventional bred varieties within five years.

1. Definition of the product variety

Based on the invention as described herein, the production of a novel potato variety based on the production of commercial hybrids is not a matter of chance, but a directed design process, starting with the articulation of the product profile of the variety. Herein, the desired product profile (target product profile) for a specific market (chips potatoes, starch industry, consumption potato must be defined. In addition, evaluation methods for the relevant traits in the product profile must be defined. Preferably, the presence of relevant traits is assessed by diagnostic markers. Such markers are well known in the art of conventional potato breeding.

2. Field trials

Depending on the season at which the development process is started, a suitable trial field is selected. When starting in September or October, it is preferred that a suitable trial field located on the southern hemisphere is selected. Field trials may start with at least 2, preferably more F3 populations, selected from at least one, but preferably more crosses of a diploid *Sli* donor and another diploid potato genotype serving as a donor for an agronomical trait, preferably the trait of vigour, which F3 populations represent the diploid, fertile, self-compatible, essentially homozygous and vigorous potato lines of the invention as exemplified in Example 2. In addition to

these F3 populations, F1 progenies are taken from at least two, preferably more selected F2 plants (as exemplified in Example 2 herein above) crossed with at least two, preferably more diploid potato genotypes that are different from the grandparents of the selected F2 plants. At least 50, preferably more individual plants of these F3 and F1 populations are then grown on the selected potato field and are selfed.

3. Evaluations and selection

During the growth in the field, the frequency of self-compatible plants that carry berries within a period of 2-6 weeks following the onset of flowering is evaluated, and at least two, preferably more F3 and F1 populations with at least 10%, preferably more self-compatible plants are selected. Further, during the growing season, the agronomic characteristics as defined in the product profile of the self-compatible plants in the selected populations is evaluated. Leaf samples of all self-compatible plants of the selected populations are collected for DNA marker testing.

At the end of the growing season the tuber quality and quantity of the self-compatible plants in the selected populations is evaluated, and the DNA marker genotypes is assessed by using diagnostic and random markers. Based on this evaluation and assessment, at least the five, preferably more, self-compatible plants of each population are selected based on: (i) the combination of traits that is closest to the target product profile; (ii) the highest level of homozygosity, and (iii) with the maximum number of contrasting homozygous loci. The selected and selfed F3 and F1 plants provide F4 and F2 progenies, respectively, in the form of seeds.

4. Tuber and seeds harvest and shipment

The tubers of the selected F3 and F1 plants as well as the F2 and F4 seeds in the berries of these selected plants are harvested at a period of at least at six weeks, preferably more, after flowering. The F4 seeds are collected for further field trials involving testing of the general combining abilities between F4 plants grown thereform, and the F2 seeds provide additional gene pools for breeding and are collected for further field trials. Depending on the season and in order to safe time, the seeds may suitably be shipped to the northern hemisphere for growing in a

greenhouse, provided that all phytosanitary regulations of the countries involved are met.

5. Tuber storage and evaluation

The tubers of the selected F1 and F3 plants are preferably stored in the region of cultivation to avoid undesired spread of pathogens and pests, and these stored tubers are evaluated for some relevant intrinsic tuber traits of the stored tubers as defined in the product profile.

6. Tuber propagation and evaluations after 1 year

The tubers of the selected F1 and F3 plants are grown the next season, preferably in the same region as the original potato field to avoid spread of unwanted pathogens or pest. At least two, preferably more potato tubers are used per plot and preferably one preferably more replicates per clone are used. At least one, preferably more relevant commercial cultivars are included as controls. Side-rows with commercial cultivars are preferably included to avoid side effects. The relevant plant characteristics as defined in the product profile (e.g. cooking, frying, chips making, starch content, etc) are evaluated. Further evaluated are the tuber yield per plot as well as the intrinsic relevant tuber characteristics as defined in the product profile. Such evaluations are used for family characterisation and progeny selections. These trials are repeated each year, preferably at locations in both hemispheres to continuously select the ancestor properties of plants, grown in the field. At later stages, when hybrids are obtained that are tested for commercial introductions, the evaluation trials are extended to at least two, preferably more, replicates at plot sizes of at least one, preferably more m^2 and at two, preferably more, locations preferably in but not restricted to the target markets.

7. Testcrosses with F4 seeds and field trials

Soon after the collection of the F4 progeny seeds of the selected selfed F3 plants, at least fifty, preferably more, of these seeds are sown. If needed, this sowing may occur in a heated greenhouse. The seedlings are preferably transplanted in small pots about 1 month after sowing and then transferred to the field in a suitable location some weeks thereafter. Evaluations and selections as described above are

continued and at least two, preferably more selected F4 plants per population are testcrossed with at least two, preferably more, other selected F4 progeny plants of two, preferably more selected parents with maximum number of contrasting loci to produce test hybrids (F4 x F4 -> hybrid). Additionally, at least two, preferably more selected F4 plants per population may be crossed with at least one, preferably more diploid genotypes with desirable characteristics or traits that are missing in the selected plants to enhance the genetic diversity of the breeding germplasm. Further, at least two, preferably more selected F4 plants per population may be selfed to produce F5 seeds. Still further, plant parts of at least two, preferably more selected F4 plants per population may be sterilised, *in vitro* propagated and treated with suitable chemicals (colchicine / oryzaline) to produce tetraploid F4 genotypes, and testcrosses are made with tetraploid F4 plants between the same parents as described above. In addition, similarly F2 progeny seeds are sown, transplanted and evaluated, crossed and selfed but not test/crossed

The above breeding scheme is repeated each year whereby an incremental increase in plant traits as defined in the product profile is achieved. The evaluations of the tubers from testcrosses are instrumental to determine the general combining abilities of parents. General combining ability is defined herein as the average performance of a line in a series of crosses. For each market at least two, preferably more elite parent lines are achieved that will render commercial hybrid seeds. It is expected that in five years a diploid or tetraploid hybrid variety is generated that is as good as or preferable better then existing commercial hybrids and that can be multiplied much faster than conventional bred varieties.

Claims

1. A diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant.

2. The potato line of claim 1, wherein said line in addition to said tuber yield comprises at least one, more preferably at least 2, 3, 4, 5 or 6 agronomically desirable traits.

3. The potato line of claim 2, wherein said at least one agronomically desirable trait is selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, and cold sweetening resistance.

4. The potato line of any one of claims 1-3, wherein plants of said line when mature attain an average fresh weight of the foliage and shoots of at least 500 grams per plant,

5. A plant of a line according to any one of claims 1-4.

6. A seed of a plant according to claim 5.

7. Seed according to claim 6, wherein said seed is a uniform hybrid potato seed.

8. A method for producing a diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, comprising:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, self-compatible, fertile and essentially homozygous potato line;

(b) providing a second potato plant, wherein said second potato plant is a plant of a diploid or tetraploid potato line which may have any level of homozygosity, said line of said second potato plant comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant;

(c) cross pollinating said first and second potato plant to provide seeds and collecting said seeds to thereby provide an offspring generation in the form of a hybrid offspring seed;

(d) growing said hybrid offspring seed into a population of hybrid offspring potato plant and selecting from said population plants producing tubers at a yield expressed in grams of fresh weight of at least 200 grams per plant, and

(e) selfing said hybrid offspring potato plant or backcrossing said hybrid offspring potato plant to a potato plant of said line of said first potato plant, for between 1 to 8 selfings or backcrosses, to thereby provide a diploid, self-compatible and essentially homozygous potato breeding line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant.

9. The method of claim 8, wherein said steps a-c are repeated and wherein step b comprises the provision of a second potato plant, wherein said second potato plant is a donor for an additional agronomically desirable trait.

10. The method according to claim 9, wherein said trait is selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, and cold sweetening resistance.

11. The method of any one of claims 8-10, wherein said first potato plant is a plant of potato line NCIMB 41663, NCIMB 41664, NCIMB 41665, or NCIMB 41765, representative seeds of said line having been deposited with the NCIMB, Aberdeen, Scotland under breeders reference AGVD1, AGVD2, AGVD3, and AGVD17 respectively.

12. The method of any one of claims 8-11, wherein said second potato plant is a plant of a second diploid, fertile, self-compatible and essentially homozygous potato line.

13. The method of any one of claims 8-11, wherein said second potato plant is a plant of a second diploid, fertile, self-incompatible potato line.

14. A diploid potato breeding line, more preferably an elite line, obtainable by the method of any one of claims 8-13.

15. A hybrid potato seed obtainable by crossing plants of a first and second diploid potato breeding line according to claim 14, wherein said plants of said first and second diploid potato breeding line contain at least 20 % contrasting homozygous loci as determinable by molecular marker analysis.

16. A method for producing a uniform hybrid potato seed, comprising:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, fertile, self-compatible and essentially homozygous potato line,

(b) providing a second potato plant, wherein said second potato plant is a plant of a second diploid, fertile, self-compatible and essentially homozygous potato line, wherein said first and second plant contain at least 20 % contrasting homozygous loci as determinable by molecular marker analysis, and

(c) cross pollinating said first and second potato plant and allowing one of said parent plants to produce berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed.

17. The method of claim 16, wherein said first and second potato plant are from potato lines comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, preferably wherein said first and second potato plant are plants having a tuber yield of at least 200 grams fresh weight per plant.

18. Method according to claim 16 or 17, wherein at least one of said first and second potato plants comprises at least one agronomically desirable trait selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, and cold sweetening resistance.

19. The method of claim 16 to 18, wherein said first potato line is a diploid, self-compatible and essentially homozygous offspring potato plant comprising the *Sli*-gene, preferably wherein said first potato plant is a plant of potato lines NCIMB 41663, NCIMB 41664, NCIMB 41665, or NCIMB 41765, representative seeds of said lines having been deposited with the NCIMB, Aberdeen, Scotland, or a diploid, self-compatible and essentially homozygous offspring potato plant thereof,.

20. The method of any one of claims 16-19, wherein said second potato plant is a plant of a second diploid, self-compatible and essentially homozygous potato line preferably said line comprising an additional agronomically desirable trait.

21. A seed of potato lines NCIMB 41663, NCIMB 41664, NCIMB 41665, or NCIMB 41765, representative seeds of said line having been deposited with the NCIMB, Aberdeen, Scotland.

22. A seed produced by the method of any one of claims 16-20.

23. A hybrid potato plant, or part thereof, produced by growing the seed of claim 16, 21 or 22.

24. Pollen or an ovule of the plant of claim 23.

25. A tuber from the plant of claim 23.

26. A potato plant having all of the physiological and morphological characteristics of the plant of claim 23.

27. A tissue culture of the plant of claim 23 or 26.

28. A potato plant regenerated from the tissue culture of claim 27, wherein said regenerated potato plant has all the physiological and morphological characteristics of a potato plant of claim 23.

29. A method for producing a hybrid potato seed wherein the method comprises crossing a first potato plant with a second potato plant and harvesting the resultant hybrid potato seed, wherein said first and/or second parent potato plant is the potato plant of claim 5, preferably wherein said first and second second parent potato have at least 20 % contrasting homozygous loci as determinable by molecular genetic marker analysis.

30. A method of producing a hybrid potato plant, said method comprising producing a seed according to a method of any one of claims 16-20 or 29 and growing said seed into a potato plant.

31. A potato plant produced by the method of claim 30, or a tuber thereof.

32. A set of at least 2 intercrossable homozygous diploid, fertile, self-compatible and essentially homozygous potato lines comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant according to anyone of claims 1-4, wherein each of said lines differ from another line in said set by having at least 20 % contrasting homozygous loci as determinable by molecular genetic marker analysis.

33. A diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant obtainable by:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, fertile, self-compatible and essentially homozygous potato line,

(b) providing a second potato plant, wherein said second potato plant is a plant of a diploid potato line having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant,

(c) cross pollinating said first and second potato plant and allowing one of said parent plants to produce berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed,

(d) growing said hybrid offspring seed into a hybrid offspring potato plant,

(e) backcrossing said hybrid offspring potato plant to a potato plant of said line of said first potato plant, and selecting from the offspring plants thus generated a plant that is diploid, self-compatible and vigorous in that it produces tubers at a yield expressed in grams of fresh weight of at least 200 grams per plant, and

(f) selfing said diploid, self-compatible and vigorous plant for between 1 to 8 generations while selecting for self-compatibility and vigour as defined in (e).

34. A hybrid potato seed which seed, when sown produces a diploid, fertile, self-compatible and essentially heterozygous potato plant having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, said plant being obtainable by:

(a) providing a first diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant,

(b) providing a second diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, wherein said first and second plant contain at least 20 % contrasting homozygous loci as determinable by molecular marker analysis, and

(c) cross pollinating said first and second potato plant and allowing at least one of said parent plants to produce berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed.

Figure 1

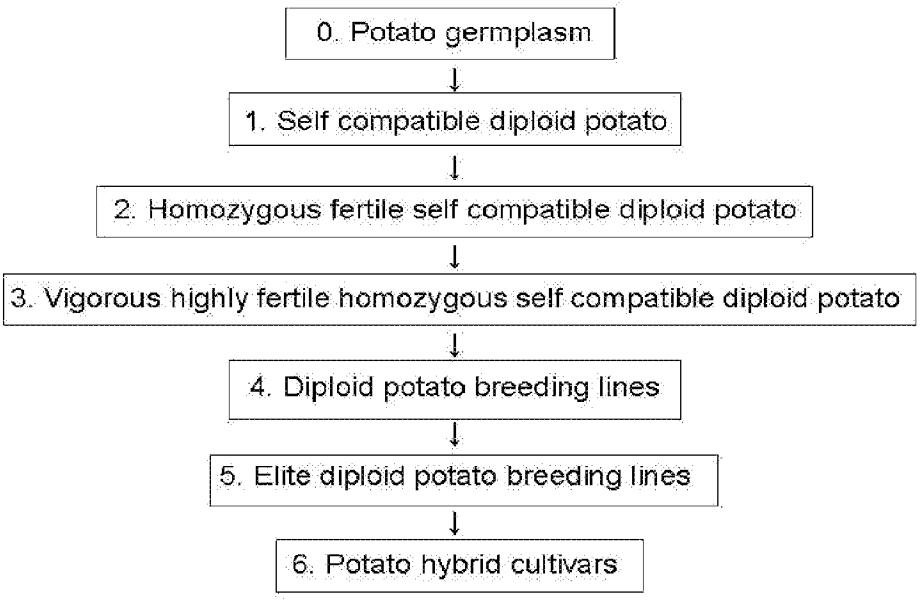

0. Potato germplasm
1. Self compatible diploid potato
2. Homozygous fertile self compatible diploid potato
3. Vigorous highly fertile homozygous self compatible diploid potato
4. Diploid potato breeding lines
5. Elite diploid potato breeding lines
6. Potato hybrid cultivars

Figure 2

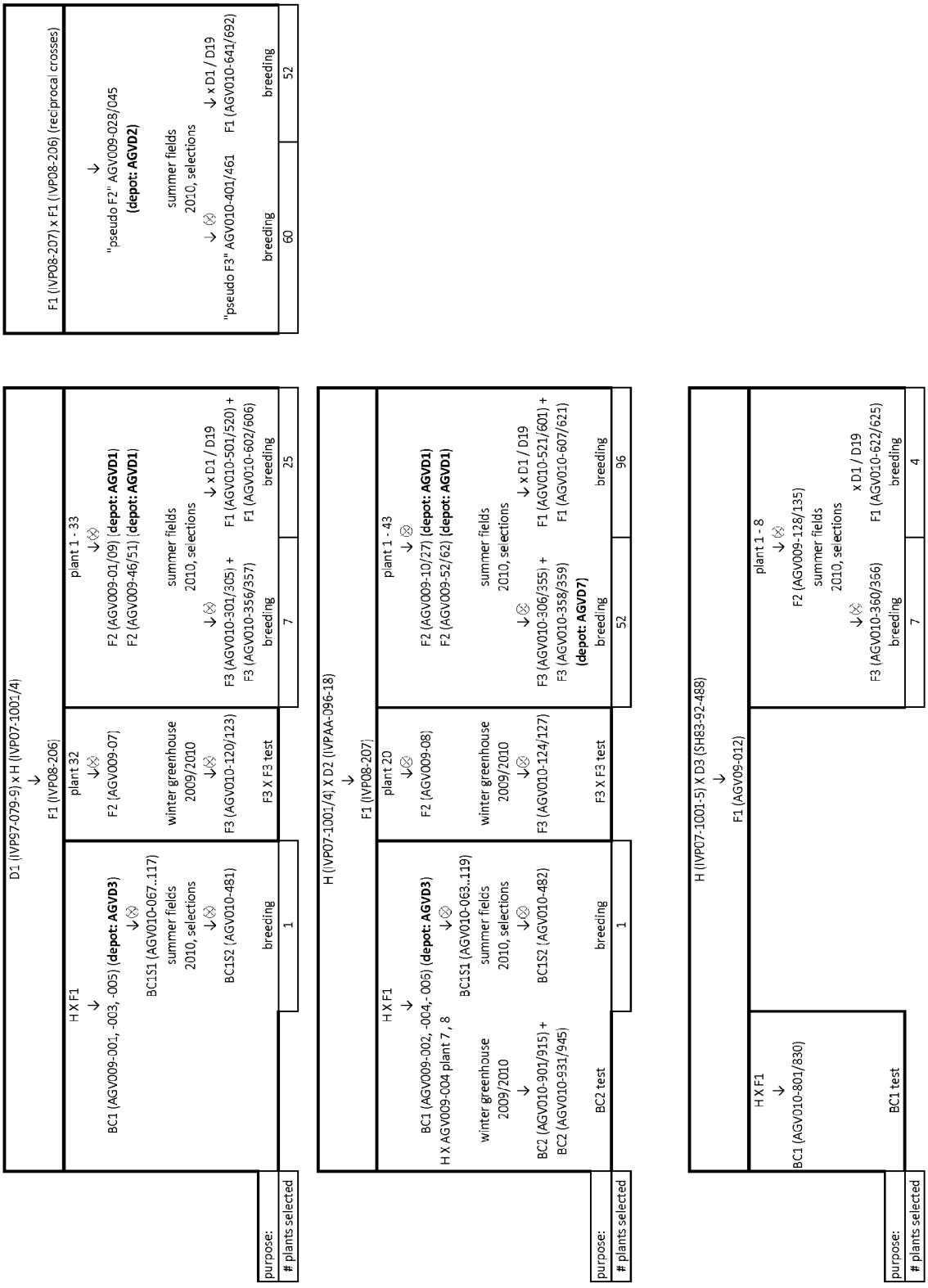

F1 (\VP08-207) x F1 (\VP08-206) (reciprocal crosses)
"pseudo F2" AGV009-028/045
(depot: AGVD2)
summer fields
2010, selections
x D1 / D19
"pseudo F3" AGV010-401/461
F1 (AGV010-641/692)
breeding
breeding
60
52
D1 (\VP97-079-9) x H (\VP07-1001/4)
F1 (\VP08-206)
H X F1
BC1 (AGV009-001, -003, -005) (depot: AGVD3)
BC1S1 (AGV010-067..117)
summer fields
2010, selections
BC1S2 (AGV010-481)
plant 32
F2 (AGV009-07)
winter greenhouse
2009/2010
F3 (AGV010-120/123)
plant 1 - 33
F2 (AGV009-01/09) (depot: AGVD1)
F2 (AGV009-46/51) (depot: AGVD1)
summer fields
2010, selections
x D1 / D19
F3 (AGV010-301/305) +
F3 (AGV010-356/357)
F1 (AGV010-501/520) +
F1 (AGV010-602/606)
breeding
breeding
F3 X F3 test
1
7
25
purpose:
plants selected
H (\VP07-1001/4) X D2 (\VPAA-096-18)
F1 (\VP08-207)
H X F1
BC1 (AGV009-002, -004,- 006) (depot: AGVD3)
H X AGV009-004 plant 7 , 8
BC1S1 (AGV010-063..119)
summer fields
2010, selections
BC1S2 (AGV010-482)
plant 20
F2 (AGV009-08)
winter greenhouse
2009/2010
F3 (AGV010-124/127)
plant 1 - 43
F2 (AGV009-10/27) (depot: AGVD1)
F2 (AGV009-52/62) (depot: AGVD1)
summer fields
2010, selections
x D1 / D19
F3 (AGV010-306/355) +
F3 (AGV010-358/359)
(depot: AGVD7)
F1 (AGV010-521/601) +
F1 (AGV010-607/621)
breeding
breeding
F3 X F3 test
1
52
96
purpose:
plants selected
H (\VP07-1001-5) X D3 (SH83-92-488)
F1 (AGV09-012)
H X F1
BC1 (AGV010-801/830)
plant 1 - 8
F2 (AGV009-128/135)
summer fields
2010, selections
x D1 / D19
F3 (AGV010-360/366)
F1 (AGV010-622/625)
breeding
breeding
BC1 test
7
4
purpose:
plants selected

Figure 3

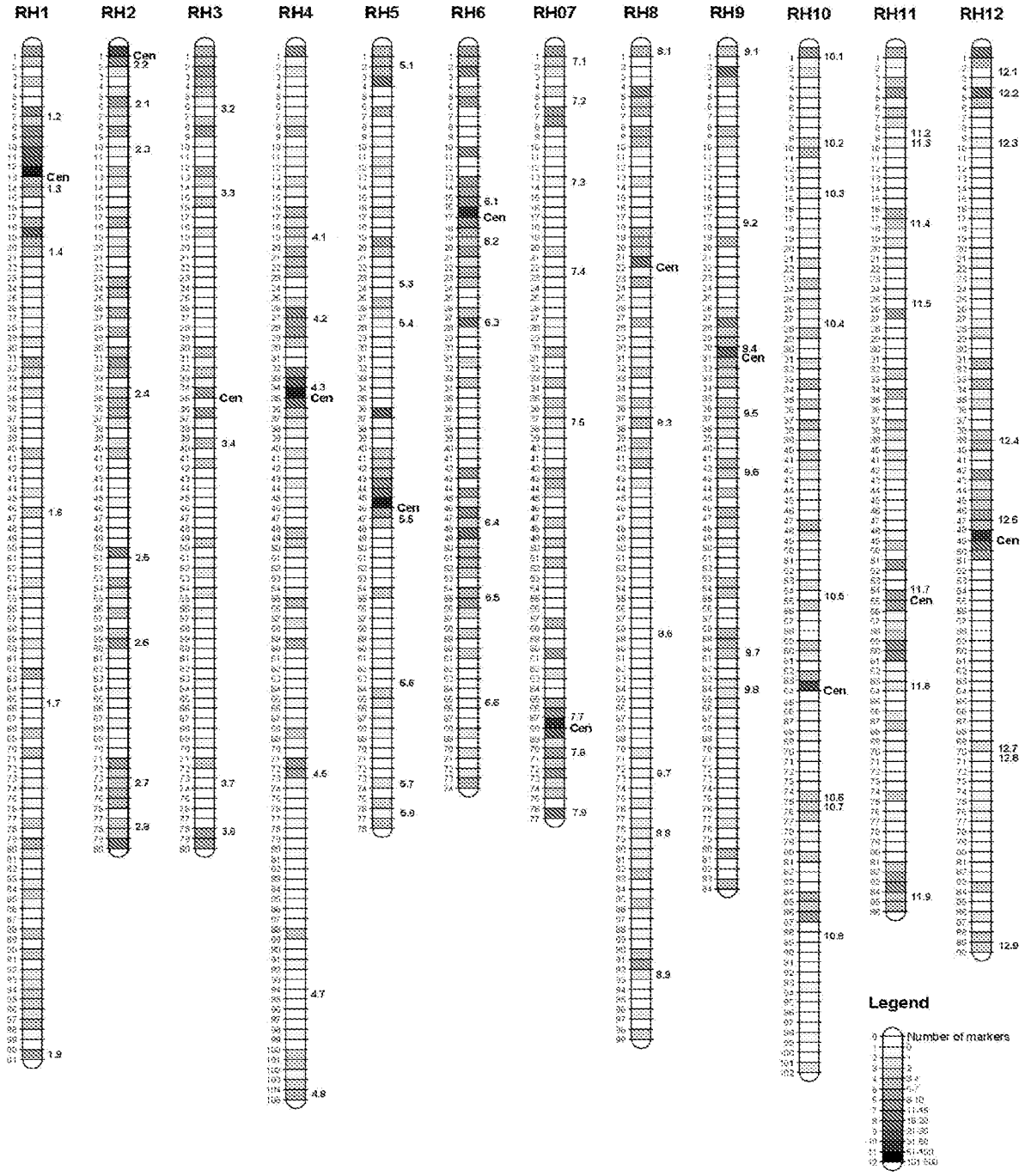

RH1
RH2
RH3
RH4
RH5
RH6
RH07
RH8
RH9
RH10
RH11
RH12
Cen
Legend
Number of markers

Figure 4

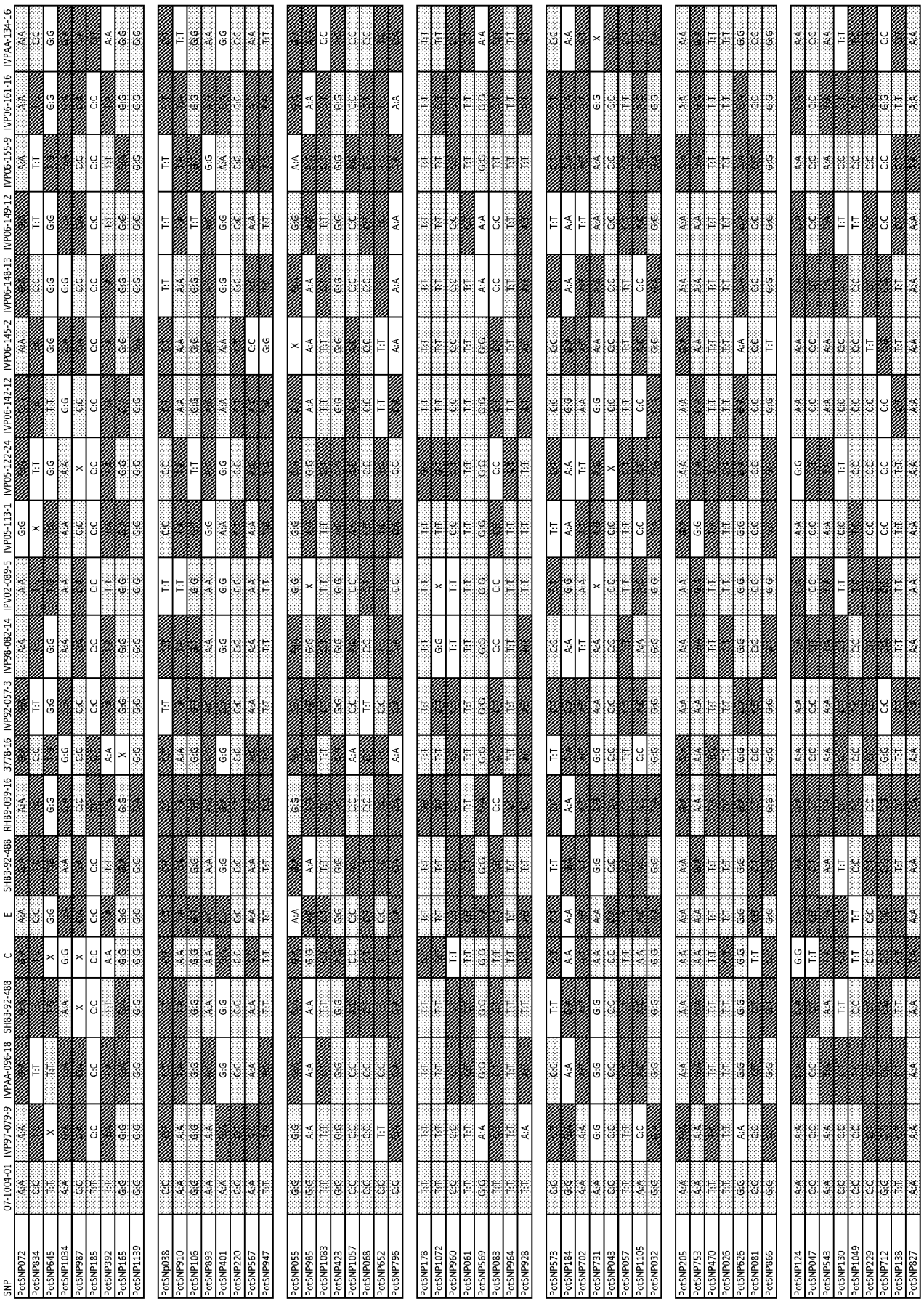
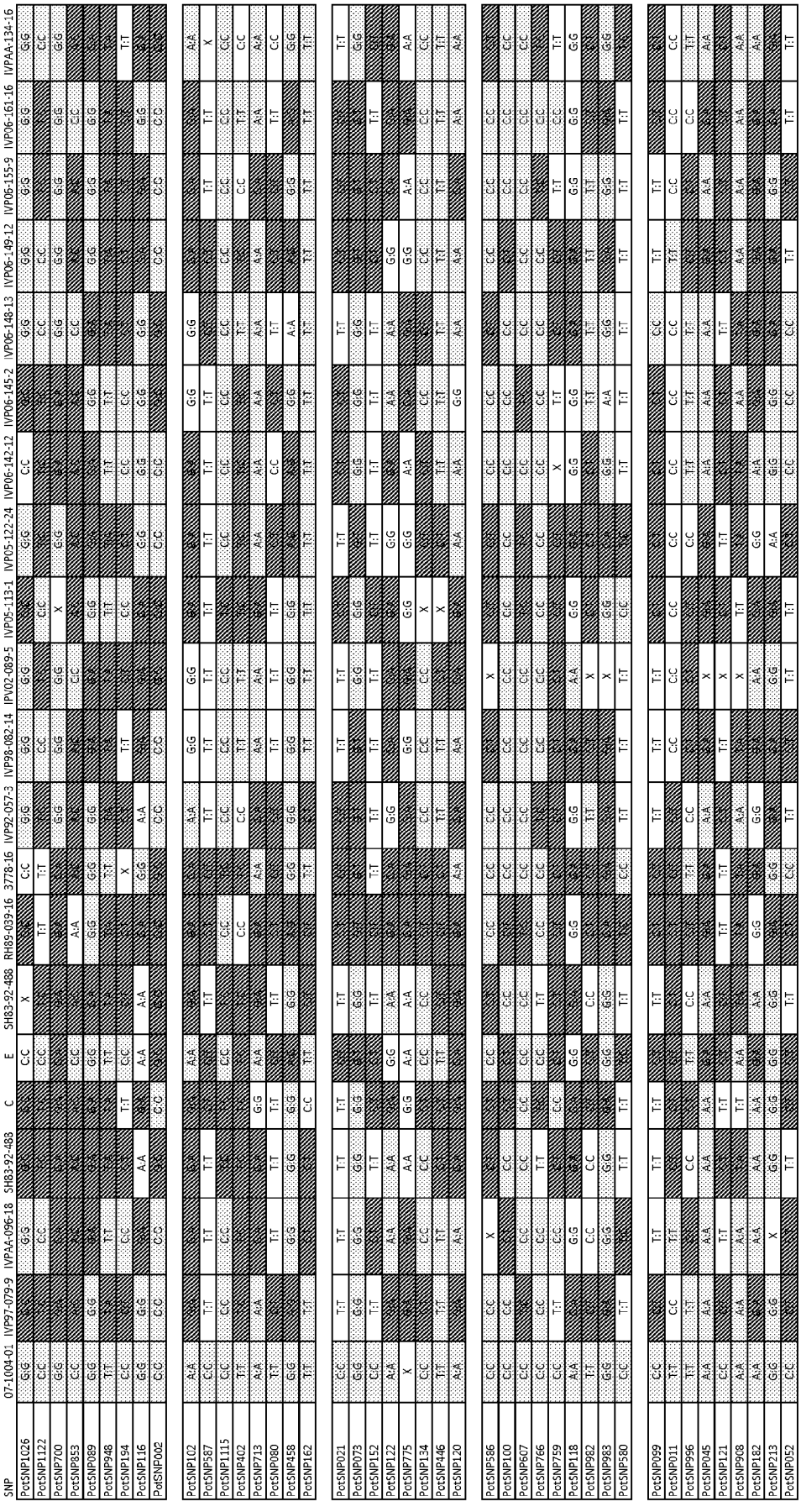

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C
E
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3778-16
IVP92-057-3
IVP98-082-14
IPV02-089-5
IVP05-113-1
IVP05-122-24
IVP06-142-12
IVP06-145-2
IVP06-148-13
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IVP06-155-9
IVP06-161-16
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PotSNP185
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PotSNP470
PotSNP026
PotSNP626
PotSNP081
PotSNP866
PotSNP124
PotSNP047
PotSNP543
PotSNP130
PotSNP1049
PotSNP229
PotSNP712
PotSNP138
PotSNP827

Figure 4 continued



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Figure 5 A

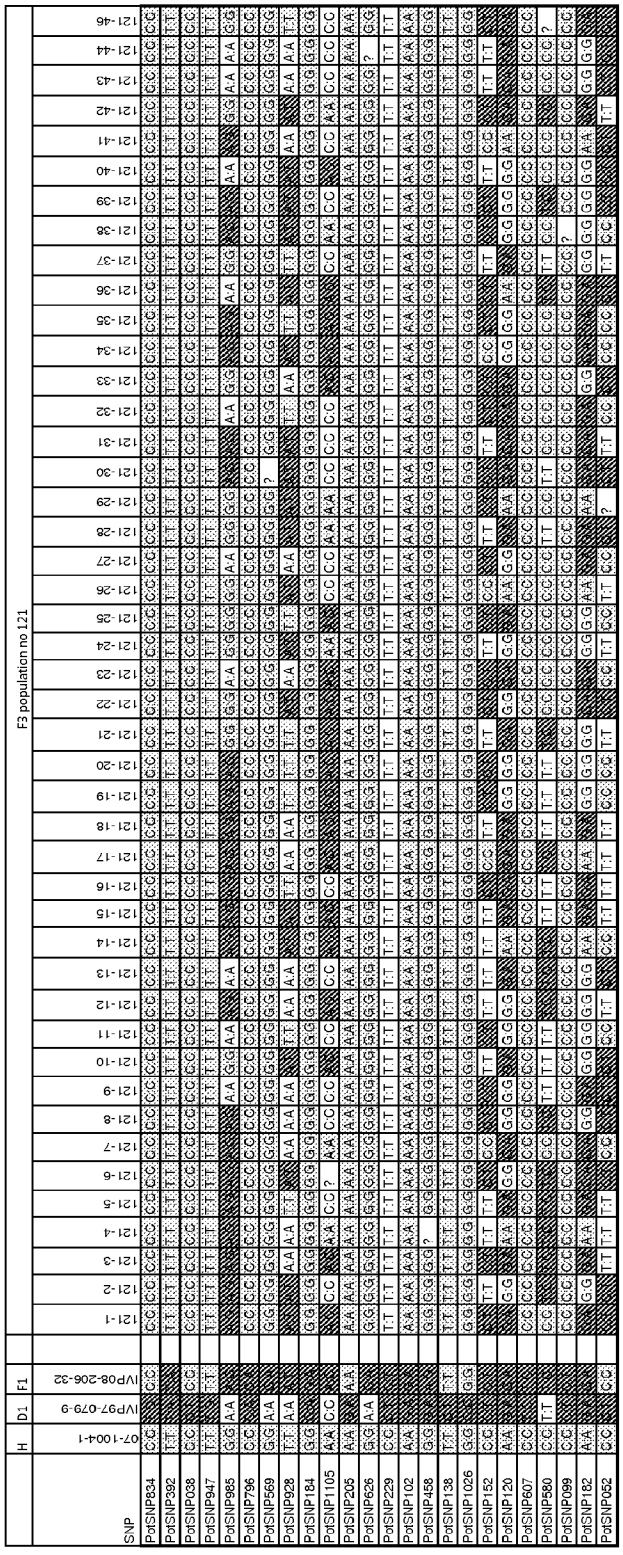

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Figure 5 B

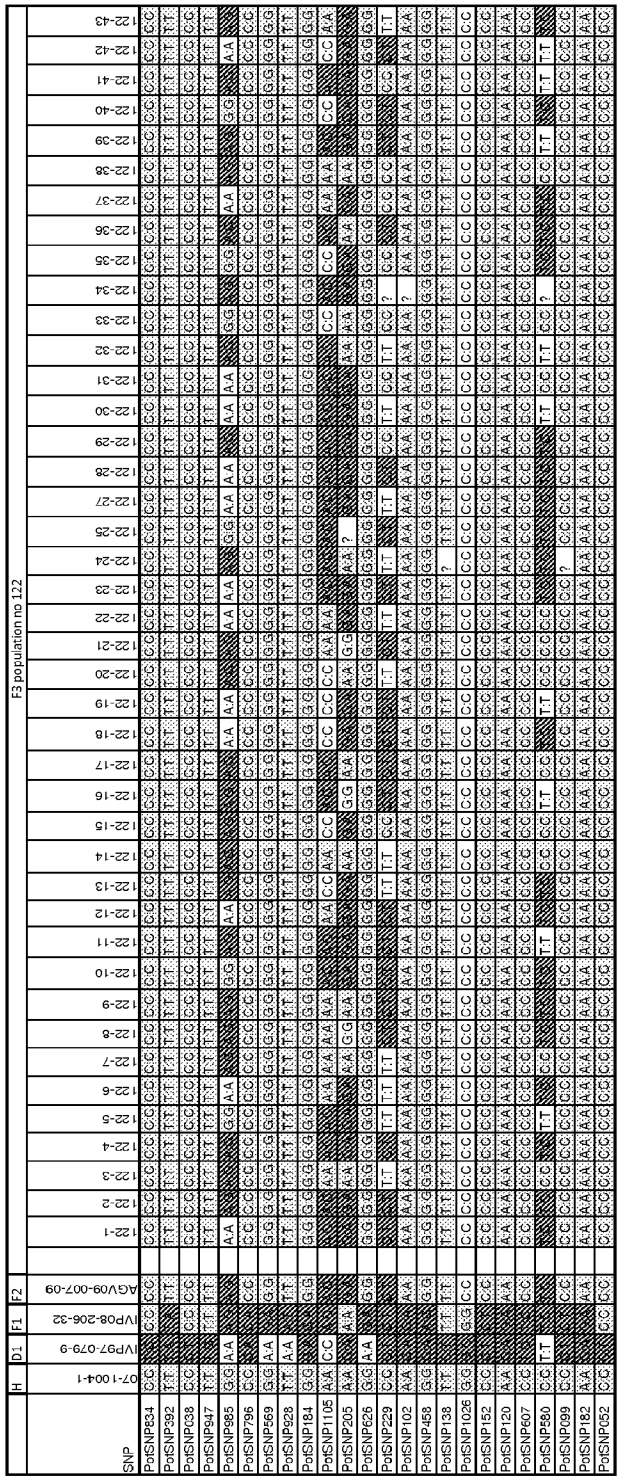

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Figure 5 C

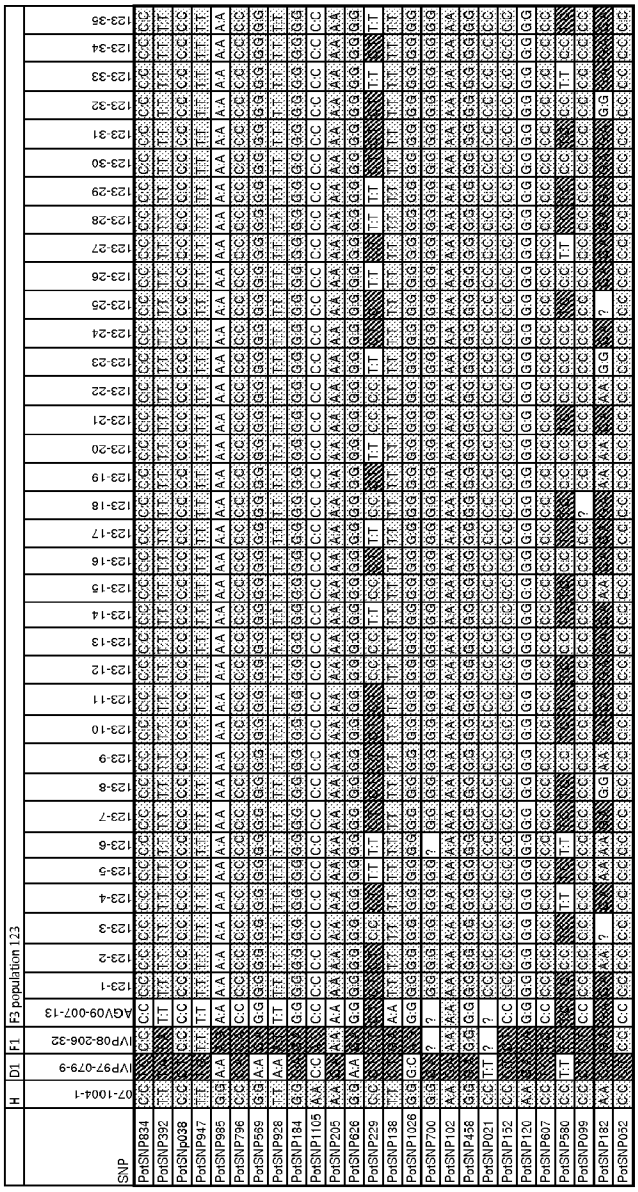

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Figure 5 D

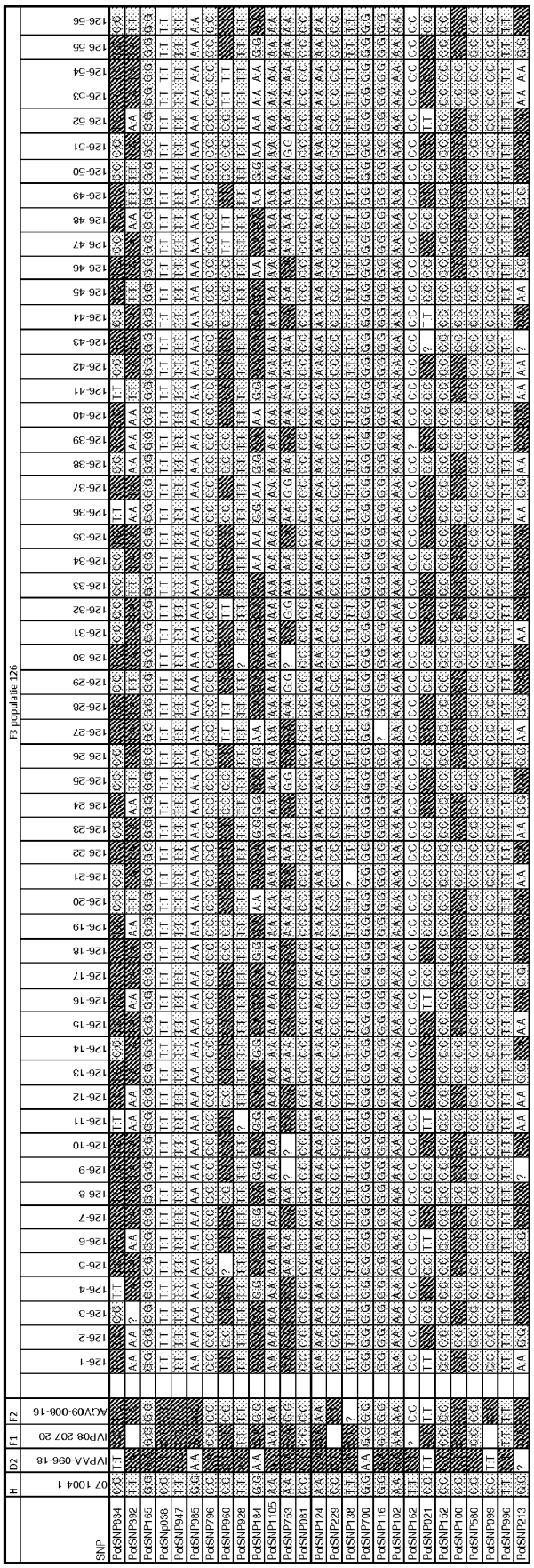

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Figure 5 E

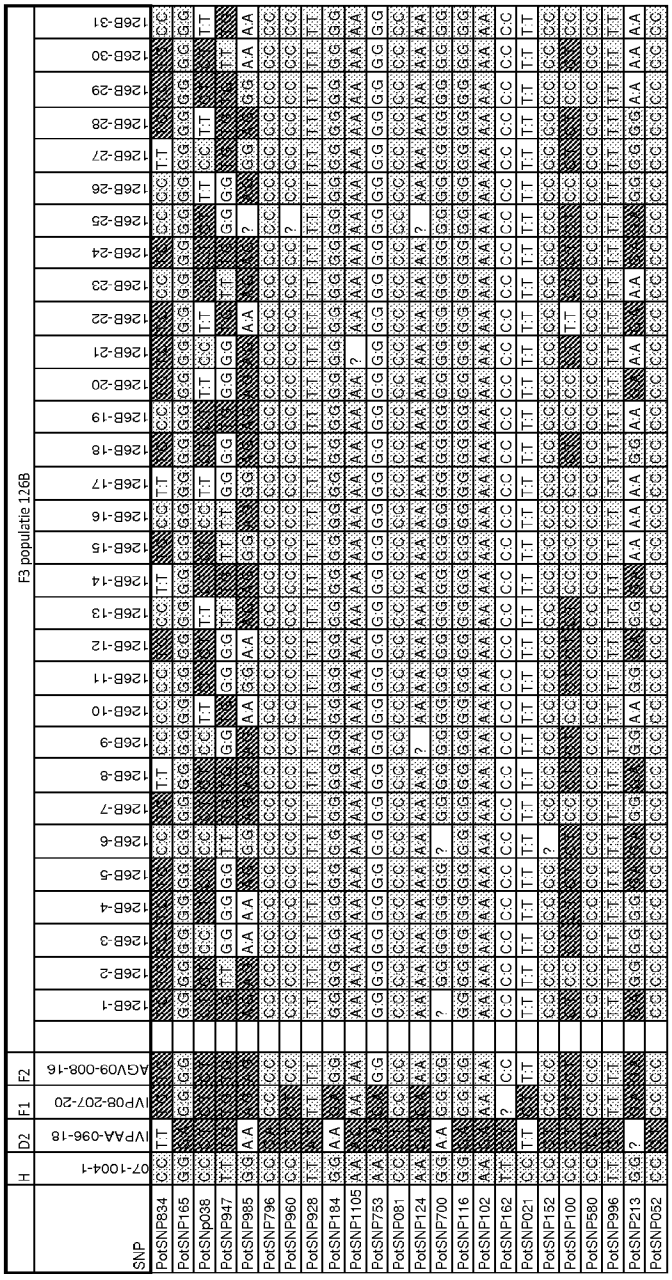
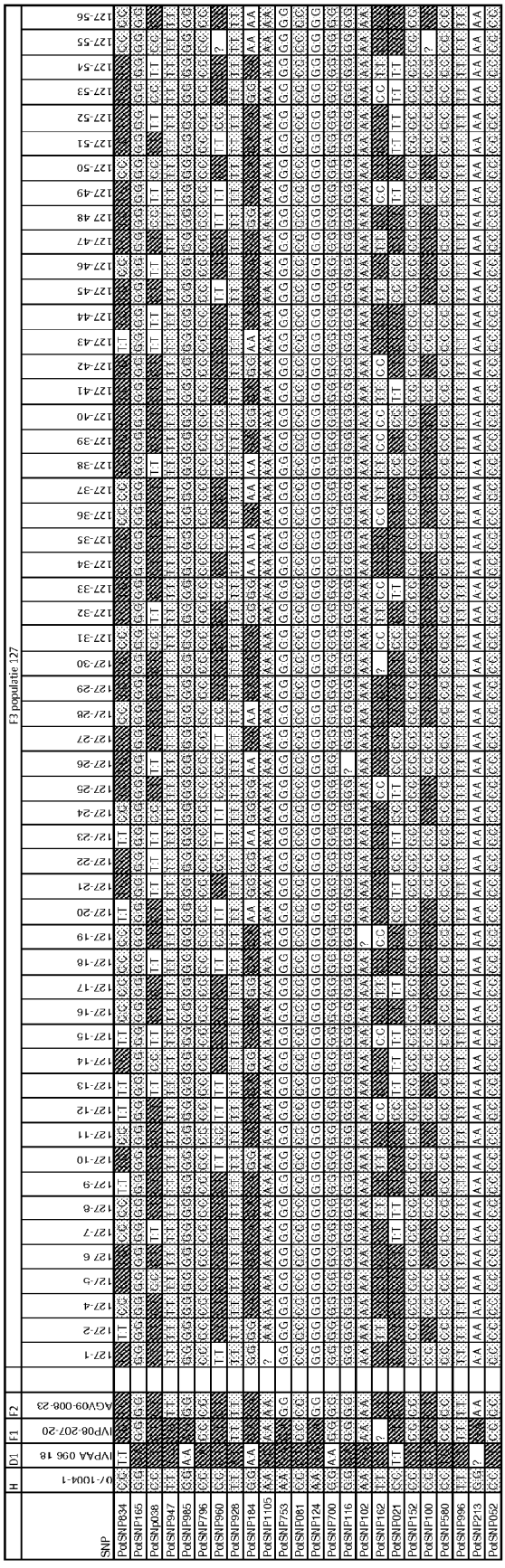

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Figure 5 F


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(74) Agent: **JANSEN, C.,M.**; Vereenigde, Johan de Wittlaan 7, NL-2517 JR Den Haag (NL).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

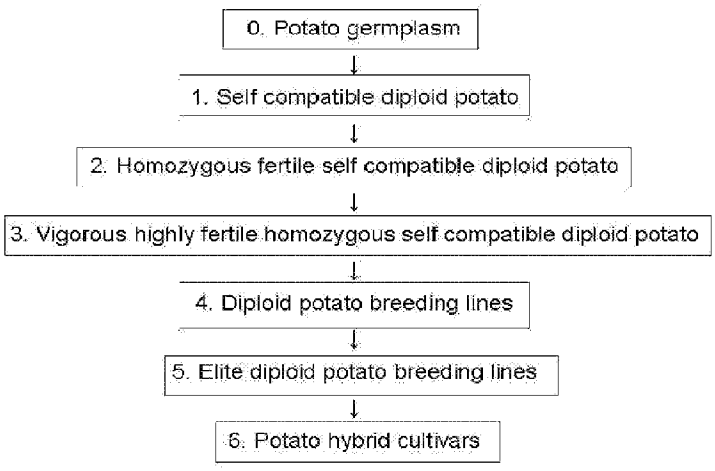
Published:

— *with international search report (Art. 21(3))*

[Continued on next page]

(54) **Title**: HYBRID SEED POTATO BREEDING

Figure 1



(57) **Abstract**: The present invention relates to diploid, fertile, self-compatible and essentially homozygous potato lines, wherein said lines comprise an agronomically desirable trait such as vigour. The invention further relates to methods for producing such plants and to hybrid seeds obtained by crossing such homozygous potato lines and to potato plants grown from said see.

WO 2011/053135 A3

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description (Rules 13bis.4(d)(i) and 48.2(a)(viii))*

(88) Date of publication of the international search report:
28 July 2011

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Willem Hendrik Lindhout *et al.*	Prior Group Art Unit: 1663
Cont. of Serial No.: 13/504,156	Prior Examiner: Russell T. Boggs
Filed: Herewith	Atty. Dkt. No.: VONL:004USC1
For: Hybrid Seed Potato Breeding	

PRELIMINARY AMENDMENT

Mail Stop Amendment
Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

The above-referenced application is a continuation of co-pending U.S. Patent Application Serial No. 13/504,156, filed July 10, 2012.

The specification of the above-referenced application is the same as that of co-pending parent application Serial No. 13/504,156, as filed, except for changes described herewith. The changes do not introduce new matter. No fees are believed due in connection with this paper. However, the Commissioner is hereby authorized to charge any fees that may become due for this filing to Dentons US LLP Account No. 19-3140/VONL:004USC1.

Specification Amendment is at page 2;

Claim Amendments begin on page 3;

Remarks begin on page 6.

SPECIFICATION AMENDMENT

Please insert the following paragraph after the title on page 1 of the application:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of United States Application No. 13/504,156, filed July 10, 2012 (pending), which application is a 371 National Stage application of PCT/NL10/50716 which application claims the benefit of priority to United States Provisional Application No. 61/254,980, filed October 26, 2009, and to European Patent Application No. 09013474.3, filed on October 26, 2009, each of which are herein incorporated by reference in their entireties.

Aardevo Exhibit 1010
Page 0109

CLAIM AMENDMENTS

Please amend the claims as follows:

1-34. (Cancelled)

35. (New) A *Solanum* species plant line, wherein said potato plant line is diploid, fertile, self-compatible, produces at least 200 grams fresh weight of tubers per plant, and at least 50% of the genomic loci in said plant line are homozygous.

36. (New) The *Solanum* species plant line of claim 35, wherein said self-compatible trait is controlled by the *Sli* gene.

37. (New) The *Solanum* species plant line of claim 35, wherein said plant line comprises at least one additional agronomically desirable traits selected from the group consisting of insect tolerance, nematode tolerance, disease resistance, herbicide tolerance, cold tolerance, drought tolerance, flooding tolerance, tolerance to dry and wet rot, salinity tolerance, yellow flesh color, and cold sweeting resistance.

38. (New) The *Solanum* species plant line of claim 35, wherein said plant line has a fresh weight of the foliage and shoots of at least 500 grams at maturity.

39. (New) A plant of the *Solanum* species plant line of claim 35.

40. (New) A seed or tuber of the plant of claim 39.

41. (New) A method of producing a *Solanum* species plant line, wherein said plant line is diploid, fertile, self-compatible, produces at least 200 grams fresh weight of tubers per plant, and at least 50% of the genomic loci in said plant line are homozygous, comprising the steps of:

(a) providing a first potato plant, wherein said first potato plant is diploid, fertile, self-compatible and essentially homozygous;

Aardevo Exhibit 1010
Page 0110

(b) providing a second potato plant, wherein said second potato plant is a diploid *Solanum* species plant, said potato plant having any level of homozygosity;

(c) cross-pollinating said first potato plant and said second potato plant to provide a seed and collecting said seed to thereby provide a hybrid offspring seed;

(d) growing a multiplicity of said hybrid offspring seed in a population of hybrid offspring potato plants and selecting from said population a self-compatible plant;

(e) inbreeding said self-compatible potato plant by selfing or backcrossing said self-compatible potato plant to a potato plant of said line of said first potato plant, for between 1 to 8 selfings or backcrosses to thereby increase the degree of homozygosity in each successive generation; and

(f) selecting within each generation of inbreeding for a fertile offspring plant to provide a diploid, fertile, self-compatible *Solanum* species plant line, wherein at least 50% of the genomic loci in said plant line are homozygous.

42. (New) The method according to claim 41, wherein said self-compatible trait is controlled by the *Sli* gene.

43. (New) The method of claim 42, wherein step (b) comprises the provision of a second potato plant, wherein said second potato plant is a donor for an additional agronomically desirable trait, wherein said trait is selected from the group consisting of insect tolerance, nematode resistance, disease resistance, herbicide tolerance, cold tolerance, drought tolerance, flooding tolerance, tolerance to dry and wet rot, salinity tolerance, yellow flesh color, and cold-sweetening resistance.

44. (New) The *Solanum* species plant line produced by the method of claim 41.

45. (New) A method of producing a uniform hybrid *Solanum* species seed comprising:

(a) providing a first *Solanum* species plant that is a first diploid, fertile, self-compatible and essentially homozygous *Solanum* potato line;

(b) providing a second *Solanum* species plant that is a diploid or tetraploid, fertile, self-compatible and essentially homozygous *Solanum* potato plant, wherein said first and second potato plant contain at least 20% contrasting homozygous loci as determined by molecular marker analysis;

(c) cross-pollinating said first and second potato plant and allowing one of said parent plants to produce berries with seeds; and

(d) collecting said seeds from said berries to provide a hybrid *Solanum* species seed.

46. (New) The method according to claim 45, wherein said self-compatible trait is controlled by the *Sli* gene.

47. (New) The method according to claim 45, wherein at least one of said first and second *Solanum* species plants comprises at least one agronomically desirable trait selected from the group consisting of insect tolerance, nematode resistance, disease resistance, herbicide tolerance, cold tolerance, drought tolerance, flooding tolerance, tolerance to dry and wet rot, salinity tolerance, yellow flesh color, and cold-sweetening resistance.

48. (New) The uniform hybrid *Solanum* species seed produced according to the method of claim 45.

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Page 0112

REMARKS

This Amendment is being filed concurrently with the filing of the instant application to update the priority and amend the claims. The claims have been amended to reflect the subject matter elected for prosecution in the current application. Specifically, claims 1-34 are cancelled herein without prejudice or disclaimer, and claims 35-48 are newly added herein. Support for the claim amendments can be found in the application and claims as originally filed. No new matter is added by the amendment.

CONCLUSION

The Examiner is invited to contact the undersigned at (214) 259-0933 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

/Ashley Hamlett/

Ashley Hamlett
Reg. No. 62,569
Agent for Applicants

DENTONS US LLP
2000 McKinney Ave., Suite 1900
Dallas, TX 75201
214-259-0933
214-259-0910 (fax)

Date: August 14, 2019

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Willem Hendrik Lindhout *et al.*	Group Art Unit: Not yet assigned
Serial No.: Filed herewith	Examiner: Not yet assigned
Filed: Filed herewith	Atty. Dkt. No.: VONL:004USC1
For: Hybrid Seed Potato Breeding	

INFORMATION DISCLOSURE STATEMENT

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Commissioner:

In compliance with the duty of disclosure under 37 C.F.R. § 1.56, it is respectfully requested that this Information Disclosure Statement be entered and the documents listed on attached Form PTO/SB/08 be considered by the Examiner and made of record. Copies of the listed documents required by 37 C.F.R. § 1.98(a)(2) are enclosed for the convenience of the Examiner.

In accordance with 37 C.F.R. §§ 1.97(g), (h), this Information Disclosure Statement is not to be construed as a representation that a search has been made, and is not to be construed to be an admission that the information cited is, or is considered to be, material to patentability as defined in 37 C.F.R. § 1.56(b).

This application is a continuation of United States Application No. 13/504,156, filed July 10, 2012 (pending), which application is a 371 National Stage application of PCT/NL10/50716 which application claims the benefit of priority to United States Provisional Application No. 61/254,980, filed October 26, 2009, and to European Patent Application No. 09013474.3, filed on October 26, 2009 and is relied upon for an earlier filing date under 35 U.S.C. § 120. In accordance with Rule 37 C.F.R. § 1.98(d), only copies of the listed documents not previously cited by or submitted to the Patent and Trademark Office in the aforementioned prior applications are enclosed.

The present Information Disclosure Statement is being filed prior to the receipt of a first Official Action reflecting an examination on the merits, and hence is believed to be timely filed in accordance with 37 C.F.R. § 1.97(b). No fees are believed to be due in connection with the filing of this Information Disclosure Statement. However, should any fees under 37 C.F.R. §§ 1.16 to 1.21 be deemed necessary for any reason relating to these materials, the Commissioner is authorized to deduct the appropriate fees from Dentons US LLP's Deposit Account No.: 19-3140/VONL:004USC1.

Respectfully submitted,

/Ashley Hamlett/

Ashley Hamlett
Reg. No. 62,569
Agent for Applicants

Dentons US LLP
2000 McKinney Avenue, Suite 1900
Dallas, Texas 75201
(214) 259-0933

Date: August 14, 2019

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
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(Not for submission under 37 CFR 1.99)

Application Number	
Filing Date	
First Named Inventor	Willem Hendrik Lindhout
Art Unit	
Examiner Name	
Attorney Docket Number	VONL:004USC1

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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	1	20140115736		2014-04-24	Lindhout et al.	

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	1	0913081	EP		1999-05-06	President of Kobe University		☐
	2	2011053135	WO		2011-05-05	Agventure B.V.		☐
	3	1289094	CA		1991-09-17	Robert D. Reichert		☐

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	Application Number	
	Filing Date	
	First Named Inventor	Willem Hendrik Lindhout
	Art Unit	
	Examiner Name	
	Attorney Docket Number	VONL:004USC1

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NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	BIRHMAN, et al., "Production of Inbred Progenies of Diploid Potatoes Using an S-locus Inhibitor (Sli) Gene, and Their Characterization", Genome, (2000) pp. 495-502, v. 43.	☐
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
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	Examiner Name	
	Attorney Docket Number	VONL:004USC1

	10	SIMKO, et al., "Assessment of Linkage Disequilibrium in Potato Genome with Single Nucleotide Polymorphism Markers", Genetics, August 2006, pp. 2237-2245, v. 173.	☐
	11	BRADSHAW, "New and Modern Methods of Potato Breeding", Chapter 8, pp. 157-77 (2007).	☐
	12	PHUMICHAI et al., "Cryptic Improvement for Fertility by Continuous Selfing of Diploid Potatoes using Sli Gene," Euphytica, 149: pp. 251-258 (2006).	☐
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
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	Examiner Name	
	Attorney Docket Number	VONL:004USC1

	21	LIPPMAN et al., Trends Genet 23(2):61-66.	☐
	22	BRADSHAW et al., (2006) Potato Res 49:49-65.	☐
	23	STOKSTAD, "The New Potato: Breeders seek a breakthrough to help farmers facing an uncertain future," Science, Vol. 363, Issue 6427, pp. 574-577, Feb. 8, 2019.	☐
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	25	HANNEMAN, "Self Fertility in Solanum." Am Potato J 62: 428-429 (1985).	☐
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	27	WANG et al., (NZ J Crop Hort Sci 28:1-8 (2000)).	☐
	28	PHUMICHAI et al., Euphytica 148:227-34 (2006).	☐
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	31	SPOONER et al., Bot Rev 80:283-383 (2014) (Year: 2014).	☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	
	Filing Date	
	First Named Inventor	Willem Hendrik Lindhout
	Art Unit	
	Examiner Name	
	Attorney Docket Number	VONL:004USC1

	32	VEILLEUX, Sol CAP News 3(2):4-4 (2011) (Year: 2011).	☐
	33	LINDHOUT et al., Potato Res 54:301-12 (2011).	☐
	34	WATANABE et al., Breed Sci 46:329-36 (1996).	☐
	35	UIJTEWAAL et al., Euphytica 36: 745-53 (1987).	☐
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	37	BAIRU et al., Plant Growth Regul 63:147-73 (2011).	☐
	38	Merriam-Webster, "as", accessed 2 December 2016.	☐
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	41	TROFIMOV et al., Translated Title: "Utilizing Diploid Varieties and Dihaploids to Intensify Potato Selection and How to Better Preserve The Viability of The Pollen of Various Species of Gourds", Translated Journal Title: Potatoes and Vegetables 6 (2007) (English translation).	☐
	42	TROFIMOV et al., "Ispol'zovaniye Diploidnykh Vidov I Digaploidov Dlya Intensifikatsii Selektsii Kartofelya And Kak Lushche Sokhranit' Zhiznesposobnost' Pylytsy Raznykh Vidov Tykvy", Kartofel' I Ovoshchi 6 (2007) (Russian).	☐

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	Attorney Docket Number	VONL:004USC1

	43	MARIS, (Euphytica 46:15-33 (1990).	☐
	44	SIMMONDS, Potato Res 40:191-214 (1997).	☐
	45	RAIMONDI et al., Euphytica 132(3):287-95 (2003).	☐
	46	DINU et al., Intl J Plant Breed Res 110(3):403-15 (2003).	☐
	47	VISKER et al., Euphytica 137(3):311-23 (2004).	☐
	48	BANI-AAMEUR et al., Euphytica 68(3):169-79 (1993).	☐
	49	International Search Report relating to corresponding PCT/NL2010/050716, issued June 8, 2011.	☐
	50	International Preliminary Report on Patentability relating to corresponding PCT/NL2010/050716, issued April 26, 2012.	☐

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	Filing Date	
	First Named Inventor	Willem Hendrik Lindhout
	Art Unit	
	Examiner Name	
	Attorney Docket Number	VONL:004USC1

CERTIFICATION STATEMENT

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☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Ashley Hamlett/	Date (YYYY-MM-DD)	2019-08-14
Name/Print	Ashley Hamlett	Registration Number	62,569

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(Not for submission under 37 CFR 1.99)

Application Number	
Filing Date	
First Named Inventor	Willem Hendrik Lindhout
Art Unit	
Examiner Name	
Attorney Docket Number	VONL:004USC1

U.S.PATENTS

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	
	Filing Date	
	First Named Inventor	Willem Hendrik Lindhout
	Art Unit	
	Examiner Name	
	Attorney Docket Number	VONL:004USC1

	1	BUSO, et al., "Tuber Yield and Quality of 4x-2x (FDR) Potato Progenies Derived from the Wild Diploid Species Solanum Berthaultii and Solanum Tarijense," Plant Breeding, 122, 229-232 (2003).	☐
	2	DeMAINE, "The Effects of Inbreeding on the Parental Values of Potato Dihaploids," Ann. Appl. Biol. (1995) 127: 151-156.	☐
	3	EIJLANDER, et al., "Selection of Vigorous and Fertile S-homo- and Heterozygous Tester Clones From Self-Incompatible Diploid Potato, Solanum Tuberosum L.," Euphytica, 97: 97-111, 1997.	☐
	4	HERMSEN, et al., "Inheritance of Genetic Markers From Two Potato Dihaploids and Their Respective Parent Cultivars," Euphytica, 27 (1978) 681-688.	☐
	5	HERMUNDSTAD, et al., "Tuber Yield and Tuber Traits of Haploid-Wild Species FI Hybrids," Potato Research 29 (1986) 289-297.	☐
	6	JANSKY, "Overcoming Hybridization Barriers in Potato," Plant Breeding, 125, 1-12 (2006).	☐
	7	PABLO, et al., "Crossability Relationships Among the Wild Diploid Potato Species Solanum Kurtzianum, S. Chacoense and S. Ruiz-Lealii from Argentina," Euphytica, 132: 2987-295, 2003.	☐
	8		☐

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	Filing Date	
	First Named Inventor	Willem Hendrik Lindhout
	Art Unit	
	Examiner Name	
	Attorney Docket Number	VONL:004USC1

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Signature	/Ashley Hamlett/	Date (YYYY-MM-DD)	2019-08-14
Name/Print	Ashley Hamlett	Registration Number	62,569

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Willem Hendrik Lindhout *et al.*	Group Art Unit: Not yet assigned
Serial No.: Filed herewith	Examiner: Not yet assigned
Filed: Filed herewith	Atty. Dkt. No.: VONL:004USC1
For: Hybrid Seed Potato Breeding	

FEE TRANSMITTAL

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Commissioner:

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Respectfully submitted,

/Ashley Hamlett/

Ashley Hamlett
Reg. No. 62,569
Agent for Applicant

Dentons US LLP
2000 McKinney Avenue, Suite 1900
Dallas, TX 75201-1858
214-259-0900
214-259-0910 (fax)

Date: August 14, 2019

Electronic Patent Application Fee Transmittal

Application Number:	16541071
Filing Date:	
Title of Invention:	Hybrid Seed Potato Breeding
First Named Inventor/Applicant Name:	Willem Hendrik Lindhout
Filer:	Ashley Greene Hamlett/Annette Armstrong
Attorney Docket Number:	VONL:004USC1

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
LATE FILING FEE FOR OATH OR DECLARATION	2051	1	80	80
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**80**

Electronic Acknowledgement Receipt

EFS ID:	36878923
Application Number:	16541071
International Application Number:	
Confirmation Number:	4348
Title of Invention:	Hybrid Seed Potato Breeding
First Named Inventor/Applicant Name:	Willem Hendrik Lindhout
Correspondence Address:	DENTONS US LLP - P.O. Box 061080 - Chicago IL 60606 US 312-876-7925 patents.us@dentons.com
Filer:	Ashley Greene Hamlett
Filer Authorized By:	
Attorney Docket Number:	VONL:004USC1
Receipt Date:	14-AUG-2019
Filing Date:	
Time Stamp:	20:12:22
Application Type:	Utility under 35 USC 111(a)

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Submitted with Payment	yes
Payment Type	CARD
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1	Miscellaneous Incoming Letter	VONL_004USC1_Fee_Transmittal.pdf	17114 8167fa1afddc6bfa9f3ecdf191169537286fd461	no	1
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REQUEST FOR TRANSFER OF A COMPUTER READABLE FORM UNDER 37 CFR 1.821(e)

Application No.:	Filed herewith	First Named Inventor:	Willem Hendrik Lindhout
Filing Date:	Filed herewith	Attorney Docket No.:	VONL:004USC1
Title of the Invention:	Hybrid Seed Potato Breeding		

The sequence information in the paper copy or PDF file of the Sequence Listing filed:

- ☑ herewith;
- ☐ as part of the originally-filed specification of this application;
- ☐ as a separate amendment filed on _____;

for the above identified application, is identical to the sequence information in the

- ☐ only
- ☑ last filed
- ☐ other (specify second, third, fourth, etc.) _____

computer readable form, which was filed on July 10, 2012,

in application number 13/504,156 filed July 10, 2012.

This computer readable form was compliant with 37 CFR 1.821-1.825, and applicant hereby requests that it be used as the computer readable form for the present application, in accordance with 37 CFR 1.821(e).

The above referenced paper copy or PDF file of the Sequence Listing contains no new matter.

A sequence listing text file submitted via EFS-Web that complies with the requirements of 37 CFR 1.824(a)(2)-(6) and (b) (i.e., is a compliant sequence listing ASCII text file), serves as both the paper copy required by 37 CFR 1.821(c) and the CRF required by 37 CFR 1.821(e). If a user submits a compliant sequence listing ASCII text file via EFS-Web, the U.S. Patent and Trademark Office will not carry out a request to use a compliant computer readable "Sequence Listing" that is already on file for another application pursuant to 37 CFR 1.821(e) but will use the sequence listing submitted with the application as originally filed via EFS-Web.

It is understood that upon the transfer of a copy of the computer readable form to this application, the U.S. Patent and Trademark Office will update the copy of the computer readable form to reflect the application number and filing date for this application.

Signature /Ashley Hamlett/	Date August 14, 2019
Name (Print/Typed) Ashley Hamlett	Registration Number 62,569

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Visser, Richard
van Eck, Herman
Hutten, Ronaldus

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Source: OPAP
Date Processed by SCORE: 08/19/2019

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Schotte, Teunis
Visser, Richard
van Eck, Herman
Hutten, Ronaldus

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Aardevo Exhibit 1010
Page 0168

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Aardevo Exhibit 1010
Page 0169

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 16/541,071

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	75		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	330		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	380		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	14 minus 20 =	*	x 50 =	0.00	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 minus 3 =	*	x 230 =	0.00			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0.00			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0.00			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	785		TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/541,071	08/14/2019	Willem Hendrik Lindhout	VONL:004USC1

CONFIRMATION NO. 4348

125771
DENTONS US LLP
P.O. Box 061080
Chicago, IL 60606

FORMALITIES LETTER

OC000000110914268

Date Mailed: 09/06/2019

NOTICE TO FILE CORRECTED APPLICATION PAPERS

Filing Date Granted

An application number and filing date have been accorded to this application. The application is informal since it does not comply with the regulations for the reason(s) indicated below. Applicant is given **TWO MONTHS** from the date of this Notice within which to correct the informalities indicated below. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

The required item(s) identified below must be timely submitted to avoid abandonment:

- A substitute specification in compliance with 37 CFR 1.52, 1.121(b)(3), and 1.125, is required. The substitute specification must be submitted with markings and be accompanied by a clean version (without markings) as set forth in 37 CFR 1.125(c) and a statement that the substitute specification contains no new matter (see 37 CFR 1.125(b)). The specification, claims, and/or abstract page(s) submitted is not acceptable and cannot be scanned or properly stored because:
  - The application papers (including any electronically submitted papers) are not in compliance with 37 CFR 1.52 because pages 63 contain text that is unreadable or of insufficient clarity.
    Application papers (including any electronically submitted papers) must be presented in a form having sufficient clarity and contrast between the background of the paper and the writing thereon to permit the Office to electronically reproduce the papers by use of digital imaging and optical character recognition. See 37 CFR 1.52(a)(1)(v).
- Replacement drawings in compliance with 37 CFR 1.84 and 37 CFR 1.121(d) are required. The drawings submitted are not acceptable because:
  - The application contains drawings and the specification contains a brief description of the drawings. However, the specification does not contain a brief description of the several views of the drawings as required by 37 CFR 1.74 and 37 CFR 1.77(b)(7) and/or a drawing(s) has not been labeled in accordance with 37 CFR 1.84(u)(1). If each figure is not labeled "Fig." with a consecutive Arabic numeral (1, 2, etc.) or an Arabic numeral and capital letter in the English alphabet (A, B, etc.), then the drawing(s) must be relabeled in accordance with 37 CFR 1.84(u)(1). In addition, if the brief description of the several views of the drawings does not refer to the figure(s) as properly labeled, then the specification must be amended to correspond to the figure(s) as properly labeled and a substitute specification in compliance with 37 CFR 1.52, 1.121(b)(3), and 1.125, is required.
  - The drawings submitted to the Office are not electronically reproducible because portions of figures 3-5F are missing and/or blurry.

- The drawings are not in compliance with 37 CFR 1.84 because figures 2, 4-5F contain figure or view numbers that have incorrect orientation. Reference characters, sheet numbers, and view numbers must be oriented in the same direction as the view. See 37 CFR 1.84(p)(1).
- The application contains a section in the specification containing the brief description of the figures, but is missing the description of Figure(s) **5A-5F** as required by 37 CFR 1.74 and 37 CFR 1.77(b)(9). A brief description of each drawing figure presented is required to satisfy this requirement.
  - To add a brief description of a figure, a proper substitute specification in compliance with 37 CFR 1.121(b)(3) and 1.125, providing a brief description of each drawing figure presented, must be submitted. See also 37 CFR 1.77(b)(9).

  Note: If applicant wishes to delete the undescribed figure, replacement sheets must be submitted to renumber the figure(s) consecutively.

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

Items Required To Avoid Processing Delays:

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):
  Willem Hendrik Lindhout

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web, including a copy of this Notice and selecting the document description "Applicant response to Pre-Exam Formalities Notice". https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/hchin/

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UNITED STATES DEPARTMENT OF COMMERCE
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
16/541,071	08/14/2019	1662	865	VONL:004USC1	14	3

CONFIRMATION NO. 4348

125771
DENTONS US LLP
P.O. Box 061080
Chicago, IL 60606

FILING RECEIPT

OC000000110914287

Date Mailed: 09/06/2019

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please submit a written request for a corrected Filing Receipt, including a properly marked-up ADS showing the changes with strike-through for deletions and underlining for additions. If you received a "Notice to File Missing Parts" or other Notice requiring a response for this application, please submit any request for correction to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections provided that the request is grantable.

Inventor(s)

Willem Hendrik Lindhout, Wageningen, NETHERLANDS;

Applicant(s)

Agventure B.V., Diepenveen, NETHERLANDS;

Power of Attorney: The patent practitioners associated with Customer Number 125771

Domestic Priority data as claimed by applicant

This application is a CON of 13/504,156 07/10/2012
which is a 371 of PCT/NL10/50716 10/26/2010
which claims benefit of 61/254,980 10/26/2009

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)
EUROPEAN PATENT OFFICE (EPO) 09013474.3 10/26/2009 No Access Code Provided

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

Request to Retrieve - This application either claims priority to one or more applications filed in an intellectual property Office that participates in the Priority Document Exchange (PDX) program or contains a proper **Request to Retrieve Electronic Priority Application(s)** (PTO/SB/38 or its equivalent). Consequently, the USPTO will attempt to electronically retrieve these priority documents.

If Required, Foreign Filing License Granted: 09/05/2019
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 16/541,071**
Projected Publication Date: To Be Determined - pending completion of Corrected Papers
Non-Publication Request: No
Early Publication Request: No
** **SMALL ENTITY** **
Title

Hybrid Seed Potato Breeding

Preliminary Class

800

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific

countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

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NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

To: patents.us@dentons.com,,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 125771

Sep 06, 2019 03:34:25 AM

Dear PAIR Customer:

DENTONS US LLP
P.O. Box 061080
Chicago, IL 60606
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 125771 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

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The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
16541071	NT.CR.APP.PA	09/06/2019	VONL:004USC1
	APP.FILE.REC	09/06/2019	VONL:004USC1

To view your correspondence online or update your email addresses, please visit us anytime at https://sportal.uspto.gov/secure/myportal/privatepair.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Willem Hendrik Lindhout *et al.*	Group Art Unit: 1662
Serial No.: 16/541,071	Examiner: Unknown
Filed: August 14, 2019	Atty. Dkt. No.: VONL:004USC1
For: Hybrid Seed Potato Breeding	Confirmation No.: 4348

REQUEST FOR CORRECTED FILING RECEIPT

Mail Stop Amendment
Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

A corrected filing receipt is requested in view of the error which appears in the filing receipt dated September 6, 2019. For the convenience of the Patent and Trademark Office, attached is a photocopy of the filing receipt on which the errors have been noted.

The errors are in regard to the listed inventors. Four inventors were not listed as inventors of this application. Please add -- Teunis Phillippus Schotte, Hazerswoude-Dorp, NL; Richard Gerardus Franciscus Visser, Bennekom, NL; Herman Johannes van Eck, Wageningen, NL; and Ronaldus Cornelis Bernardus Hutte, Rhenen, NL-- as inventors of this application. In support of the requested corrections, the Applicant attaches a copy of the Application Data Sheet as submitted on August 14, 2019.

No fees are believed to be due in connection with the filing of this document. However, should any fee under 37 C.F.R. §§ 1.16 to 1.21 be deemed necessary for any

reason related to this document, the Commissioner is hereby authorized to deduct the appropriate fee from Dentons US LLP Account No. 19-3140/VONL:004USC1.

Respectfully submitted,

/Ashley Hamlett/

Ashley Hamlett
Reg. No. 62,569
Agent for Applicants

DENTONS US LLP
2000 McKinney Ave., Suite 1900
Dallas, TX 75201
214-259-0933
214-259-0910 (fax)

Date: October 17, 2019

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
16/541,071	08/14/2019	1662	865	VONL:004USC1	14	3

CONFIRMATION NO. 4348

125771
DENTONS US LLP
P.O. Box 061080
Chicago, IL 60606

FILING RECEIPT

OC000000110914287

Date Mailed: 09/06/2019

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Inventor(s)

Willem Hendrik Lindhout, Wageningen, NETHERLANDS; → Teunis Phillipus Schotte, Hazerswoude-Dorp, NL
Richard Gerardus Franciscus Visser, Bennekom, NL
Herman Johannes van Eck, Wageningen, NL
Ronaldus Cornelis Bernadus Hutten, Rhenen, NL

Applicant(s)

Agventure B.V., Diepenveen, NETHERLANDS;

Power of Attorney: The patent practitioners associated with Customer Number 125771

Domestic Priority data as claimed by applicant

This application is a CON of 13/504,156 07/10/2012
which is a 371 of PCT/NL10/50716 10/26/2010
which claims benefit of 61/254,980 10/26/2009

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)
EUROPEAN PATENT OFFICE (EPO) 09013474.3 10/26/2009 No Access Code Provided

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

Request to Retrieve - This application either claims priority to one or more applications filed in an intellectual property Office that participates in the Priority Document Exchange (PDX) program or contains a proper **Request to Retrieve Electronic Priority Application(s)** (PTO/SB/38 or its equivalent). Consequently, the USPTO will attempt to electronically retrieve these priority documents.

If Required, Foreign Filing License Granted: 09/05/2019
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 16/541,071**
Projected Publication Date: To Be Determined - pending completion of Corrected Papers
Non-Publication Request: No
Early Publication Request: No
** **SMALL ENTITY** **
Title

Hybrid Seed Potato Breeding

Preliminary Class

800

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

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countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	VONL:004USC1
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Willem	Hendrik	Lindhout	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Wageningen	Country of Residence	NL

Mailing Address of Inventor:

Address 1	Delhorstpad 1A		
Address 2			
City	Wageningen	**State/Province**	
Postal Code	6703 BE	**Country**	NL

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Teunis	Philippus	Schotte	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Hazerswoude-Dorp	Country of Residence	NL

Mailing Address of Inventor:

Address 1	Westzijdeweg 33A		
Address 2			
City	Hazerswoude-Dorp	**State/Province**	
Postal Code	2391 JE	**Country**	NL

Inventor 3 Remove

Legal Name

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	VONL:004USC1
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

Prefix	Given Name	Middle Name	Family Name	Suffix
	Richard	Gerardus Franciscus	Visser	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Bennekom	Country of Residence	NL

Mailing Address of Inventor:

Address 1	Emmalaan 17		
Address 2			
City	Bennekom	State/Province	
Postal Code	6721 ET	Country	NL

Inventor 4 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Herman	Johannes	van Eck	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Wageningen	Country of Residence	NL

Mailing Address of Inventor:

Address 1	Eykmanstraat 9		
Address 2			
City	Wageningen	State/Province	
Postal Code	6706 JT	Country	NL

Inventor 5 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Ronaldus	Cornelis Bernardus	Hutten	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Rhenen	Country of Residence	NL

Mailing Address of Inventor:

Address 1	DeThijmenstoren 12		
Address 2			
City	Rhenen	State/Province	
Postal Code	3912 AT	Country	NL

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	VONL:004USC1
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	125771		
Email Address		Add Email	Remove Email

Application Information:

Title of the Invention	Hybrid Seed Potato Breeding		
Attorney Docket Number	VONL:004USC1	**Small Entity Status Claimed**	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	11	**Suggested Figure for Publication (if any)**	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	VONL:004USC1
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	125771		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Continuation of	13504156	2012-07-10
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
13504156	a 371 of international	PCT/NL10/50716	2010-10-26
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/NL10/50716	Claims benefit of provisional	61254980	2009-10-26

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

			Remove
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)
09013474.3	EP	2009-10-26	

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

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	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	VONL:004USC1
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	VONL:004USC1
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◉ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
○ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	Agventure B.V.

Mailing Address Information For Applicant:

Address 1	Burg. Arriensweg 32		
Address 2			
City	Diepenveen	**State/Province**	
Country	NL	Postal Code	7431 BM
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button.

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	VONL:004USC1
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Ashley Hamlett/			Date (YYYY-MM-DD)	2019-08-14
First Name	Ashley	Last Name	Hamlett	Registration Number	62569

Additional Signature may be generated within this form by selecting the Add button.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	VONL:004USC1
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Willem Hendrik Lindhout *et al.*	Group Art Unit: 1662
Serial No.: 16/541,071	Examiner: Unknown
Filed: August 14, 2019	Atty. Dkt. No.: VONL:004USC1
For: Hybrid Seed Potato Breeding	Confirmation No.: 4348

RESPONSE TO NOTICE TO FILE CORRECTED APPLICATION PAPERS

MS MISSING PARTS
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Commissioner:

In response to the Notice to File Corrected Application Papers, dated September 6, 2019, enclosed are the following:

(a) Replacement Drawings;

(b) Two Substitute Specifications in marked and unmarked form. The Substitute Specifications are being submitted to add the priority paragraph after the title on page one of the specification; to correct the Brief Description of Drawings in the originally filed specification; to replace table 5A beginning on page 53 of the originally filed specification; and to replace the table on page 63 of the originally filed specification due to insufficient clarity, support for the amendments can be found in the application and sequence listing as filed, no new matter is added;

(c) A copy of Notice to File Corrected Application Papers.

Aardevo Exhibit 1010
Page 0194

No fees are believed to be due in connection with the filing of this document. However, should any fee under 37 C.F.R. §§ 1.16 to 1.21 be deemed necessary for any reason related to this document, the Commissioner is hereby authorized to deduct the appropriate fee from Dentons US LLP Account No. 19-3140/VONL:004USC1.

.

Respectfully submitted,

/Ashley Hamlett/

Ashley Hamlett
Reg. No. 62,569
Agent for Applicants

Dentons US LLP
2000 McKinney Avenue, Suite 1900
Dallas, Texas 75201
(214) 259-0933

Date: October 17, 2019

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/541,071	08/14/2019	Willem Hendrik Lindhout	VONL:004USC1

CONFIRMATION NO. 4348

125771
DENTONS US LLP
P.O. Box 061080
Chicago, IL 60606

FORMALITIES LETTER

OC000000110914268

Date Mailed: 09/06/2019

NOTICE TO FILE CORRECTED APPLICATION PAPERS

Filing Date Granted

An application number and filing date have been accorded to this application. The application is informal since it does not comply with the regulations for the reason(s) indicated below. Applicant is given **TWO MONTHS** from the date of this Notice within which to correct the informalities indicated below. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

The required item(s) identified below must be timely submitted to avoid abandonment:

- A substitute specification in compliance with 37 CFR 1.52, 1.121(b)(3), and 1.125, is required. The substitute specification must be submitted with markings and be accompanied by a clean version (without markings) as set forth in 37 CFR 1.125(c) and a statement that the substitute specification contains no new matter (see 37 CFR 1.125(b)). The specification, claims, and/or abstract page(s) submitted is not acceptable and cannot be scanned or properly stored because:
  - The application papers (including any electronically submitted papers) are not in compliance with 37 CFR 1.52 because pages 63 contain text that is unreadable or of insufficient clarity.
    Application papers (including any electronically submitted papers) must be presented in a form having sufficient clarity and contrast between the background of the paper and the writing thereon to permit the Office to electronically reproduce the papers by use of digital imaging and optical character recognition. See 37 CFR 1.52(a)(1)(v).
- Replacement drawings in compliance with 37 CFR 1.84 and 37 CFR 1.121(d) are required. The drawings submitted are not acceptable because:
  - The application contains drawings and the specification contains a brief description of the drawings. However, the specification does not contain a brief description of the several views of the drawings as required by 37 CFR 1.74 and 37 CFR 1.77(b)(7) and/or a drawing(s) has not been labeled in accordance with 37 CFR 1.84(u)(1). If each figure is not labeled "Fig." with a consecutive Arabic numeral (1, 2, etc.) or an Arabic numeral and capital letter in the English alphabet (A, B, etc.), then the drawing(s) must be relabeled in accordance with 37 CFR 1.84(u)(1). In addition, if the brief description of the several views of the drawings does not refer to the figure(s) as properly labeled, then the specification must be amended to correspond to the figure(s) as properly labeled and a substitute specification in compliance with 37 CFR 1.52, 1.121(b)(3), and 1.125, is required.
  - The drawings submitted to the Office are not electronically reproducible because portions of figures 3-5F are missing and/or blurry.

- The drawings are not in compliance with 37 CFR 1.84 because figures 2, 4-5F contain figure or view numbers that have incorrect orientation. Reference characters, sheet numbers, and view numbers must be oriented in the same direction as the view. See 37 CFR 1.84(p)(1).
- The application contains a section in the specification containing the brief description of the figures, but is missing the description of Figure(s) **5A-5F** as required by 37 CFR 1.74 and 37 CFR 1.77(b)(9). A brief description of each drawing figure presented is required to satisfy this requirement.
  - To add a brief description of a figure, a proper substitute specification in compliance with 37 CFR 1.121(b)(3) and 1.125, providing a brief description of each drawing figure presented, must be submitted. See also 37 CFR 1.77(b)(9).

  Note: If applicant wishes to delete the undescribed figure, replacement sheets must be submitted to renumber the figure(s) consecutively.

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

Items Required To Avoid Processing Delays:

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):
  Willem Hendrik Lindhout

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web, including a copy of this Notice and selecting the document description "Applicant response to Pre-Exam Formalities Notice". https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/hchin/

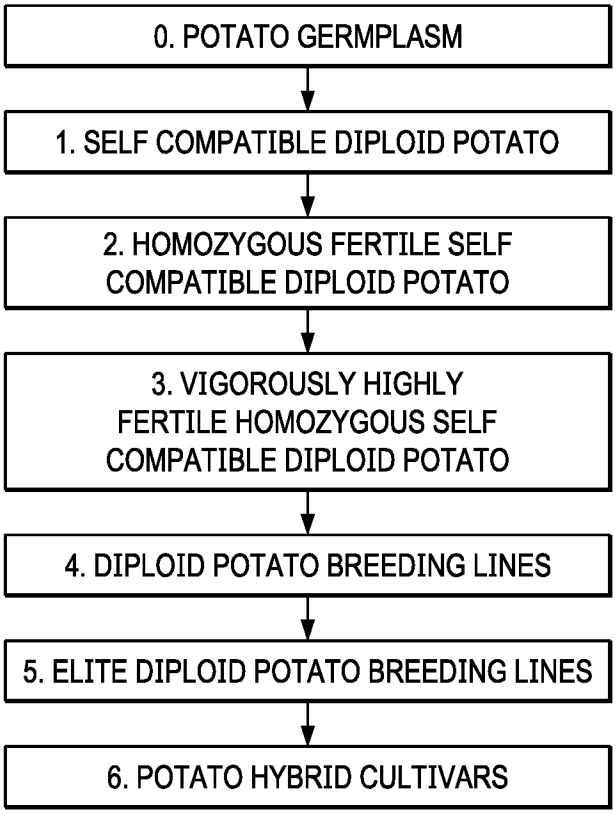
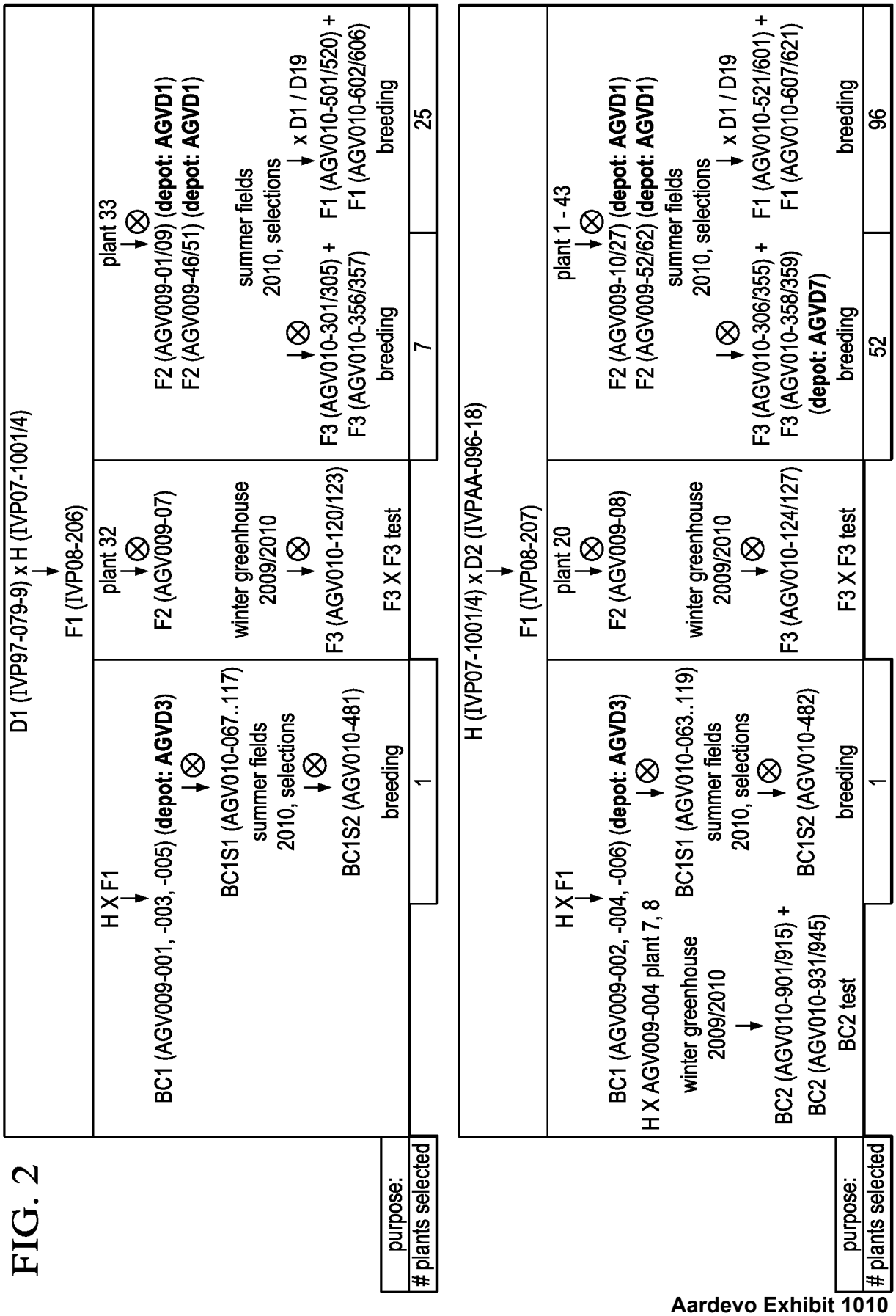

0. POTATO GERMPLASM
1. SELF COMPATIBLE DIPLOID POTATO
2. HOMOZYGOUS FERTILE SELF COMPATIBLE DIPLOID POTATO
3. VIGOROUSLY HIGHLY FERTILE HOMOZYGOUS SELF COMPATIBLE DIPLOID POTATO
4. DIPLOID POTATO BREEDING LINES
5. ELITE DIPLOID POTATO BREEDING LINES
6. POTATO HYBRID CULTIVARS

FIG. 1

FIG. 2


D1 (IVP97-079-9) x H (IVP07-1001/4)
F1 (IVP08-206)
H X F1
BC1 (AGV009-001, -003, -005) (depot: AGVD3)
BC1S1 (AGV010-067..117)
summer fields
2010, selections
BC1S2 (AGV010-481)
breeding
plant 32
F2 (AGV009-07)
winter greenhouse
2009/2010
F3 (AGV010-120/123)
F3 X F3 test
plant 33
F2 (AGV009-01/09) (depot: AGVD1)
F2 (AGV009-46/51) (depot: AGVD1)
summer fields
2010, selections
x D1 / D19
F3 (AGV010-301/305) + F1 (AGV010-501/520) +
F3 (AGV010-356/357) F1 (AGV010-602/606)
breeding
breeding
purpose:
plants selected
1
7
25
H (IVP07-1001/4) x D2 (IVPAA-096-18)
F1 (IVP08-207)
H X F1
BC1 (AGV009-002, -004, -006) (depot: AGVD3)
H X AGV009-004 plant 7, 8
winter greenhouse
2009/2010
BC2 (AGV010-901/915) +
BC2 (AGV010-931/945)
BC2 test
BC1S1 (AGV010-063..119)
summer fields
2010, selections
BC1S2 (AGV010-482)
breeding
plant 20
F2 (AGV009-08)
winter greenhouse
2009/2010
F3 (AGV010-124/127)
F3 X F3 test
plant 1 - 43
F2 (AGV009-10/27) (depot: AGVD1)
F2 (AGV009-52/62) (depot: AGVD1)
summer fields
2010, selections
x D1 / D19
F3 (AGV010-306/355) + F1 (AGV010-521/601) +
F3 (AGV010-358/359) F1 (AGV010-607/621)
(depot: AGVD7)
breeding
breeding
purpose:
plants selected
1
52
96

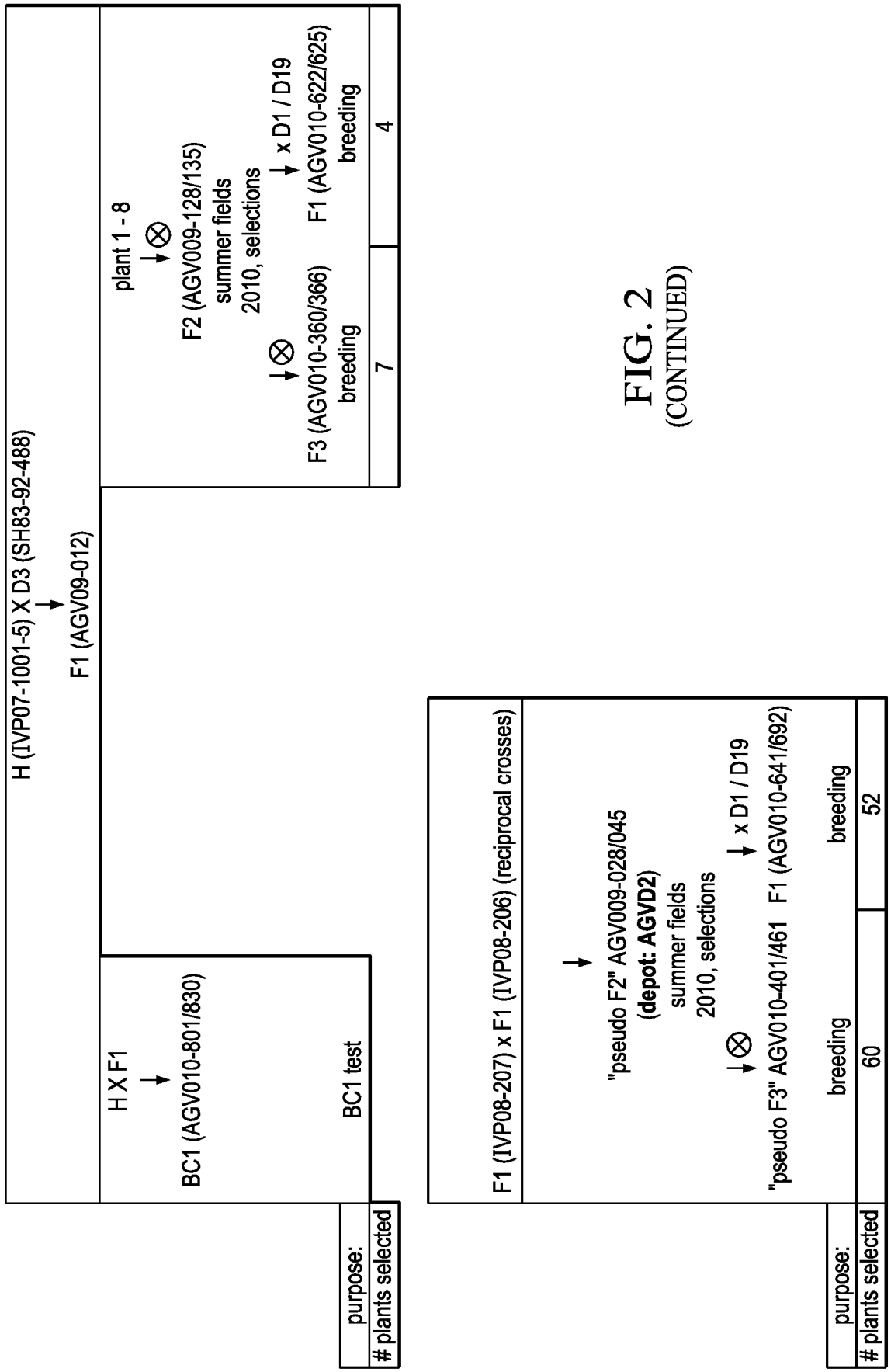


FIG. 2
(CONTINUED)

REPLACEMENT SHEET

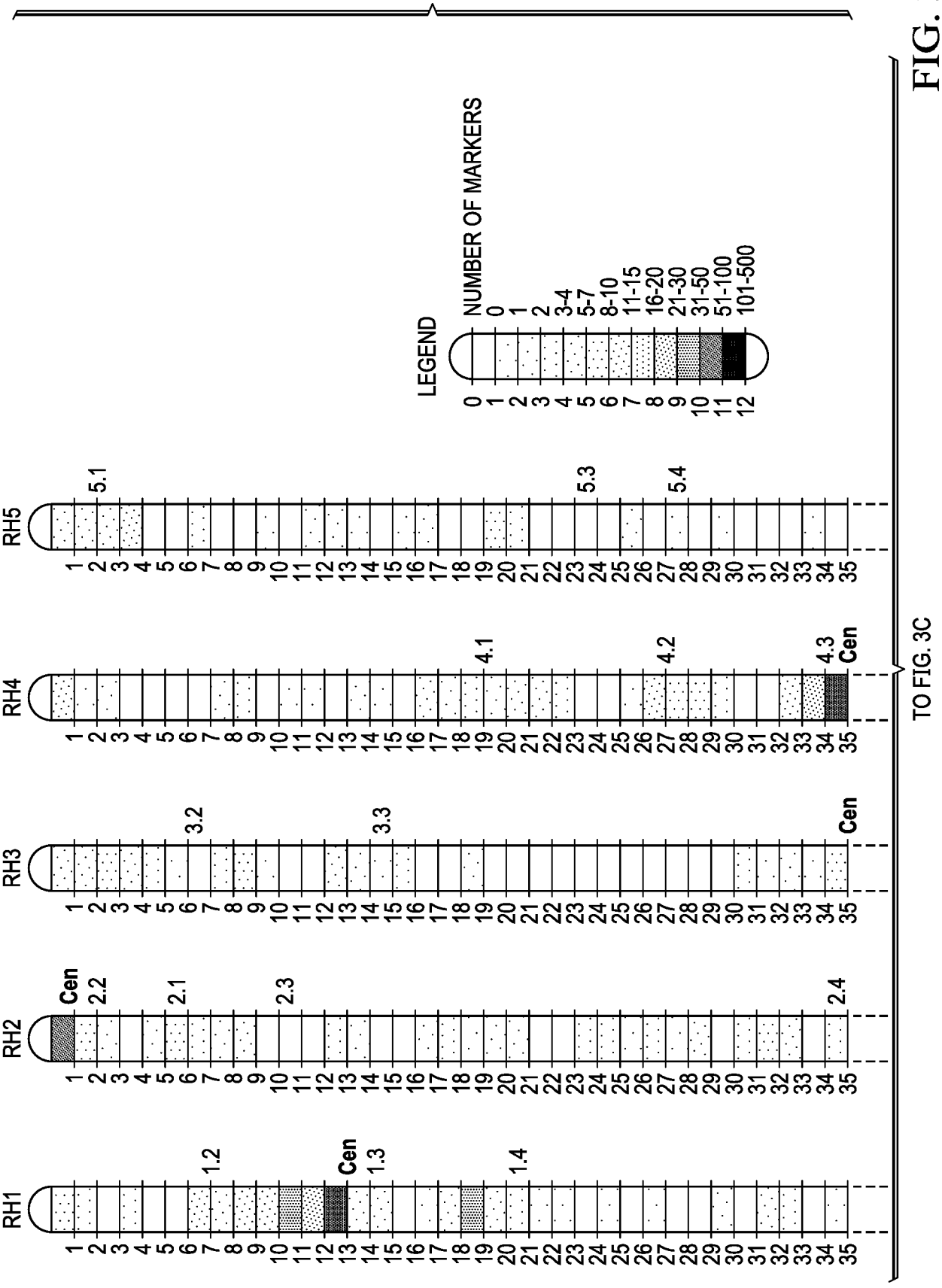


FIG. 3A

REPLACEMENT
SHEET

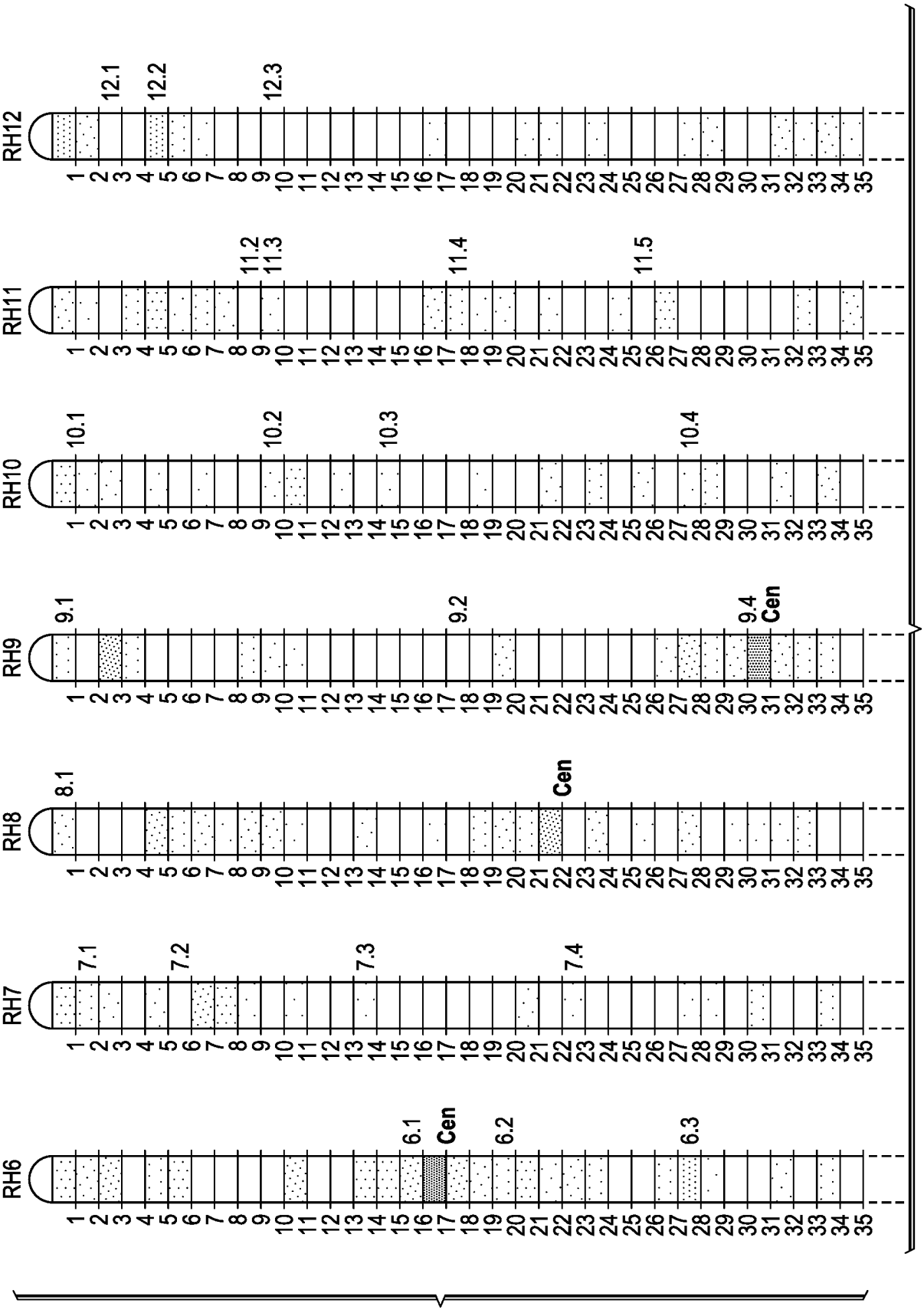


FIG. 3B

REPLACEMENT
SHEET

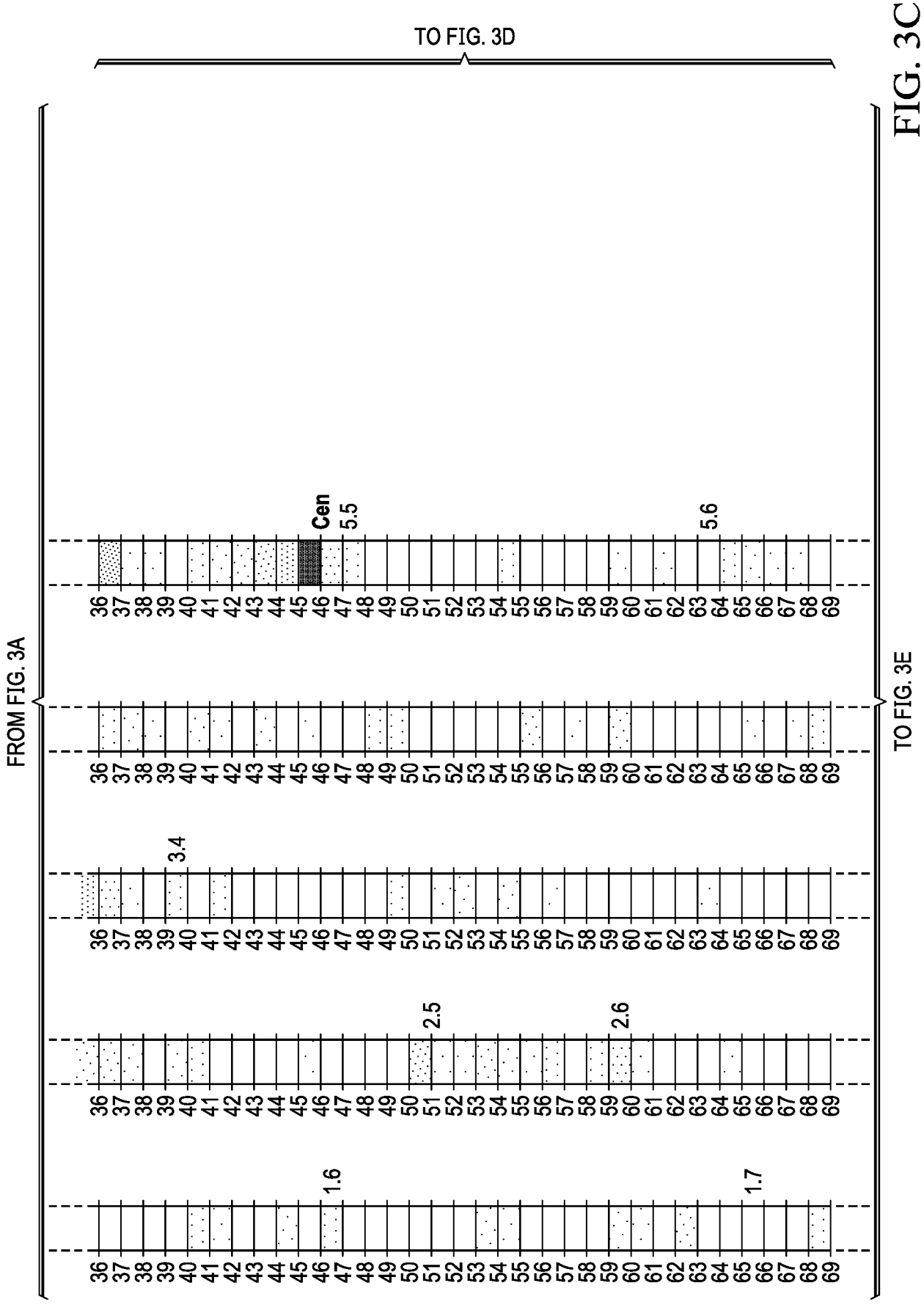


FIG. 3C

REPLACEMENT
SHEET

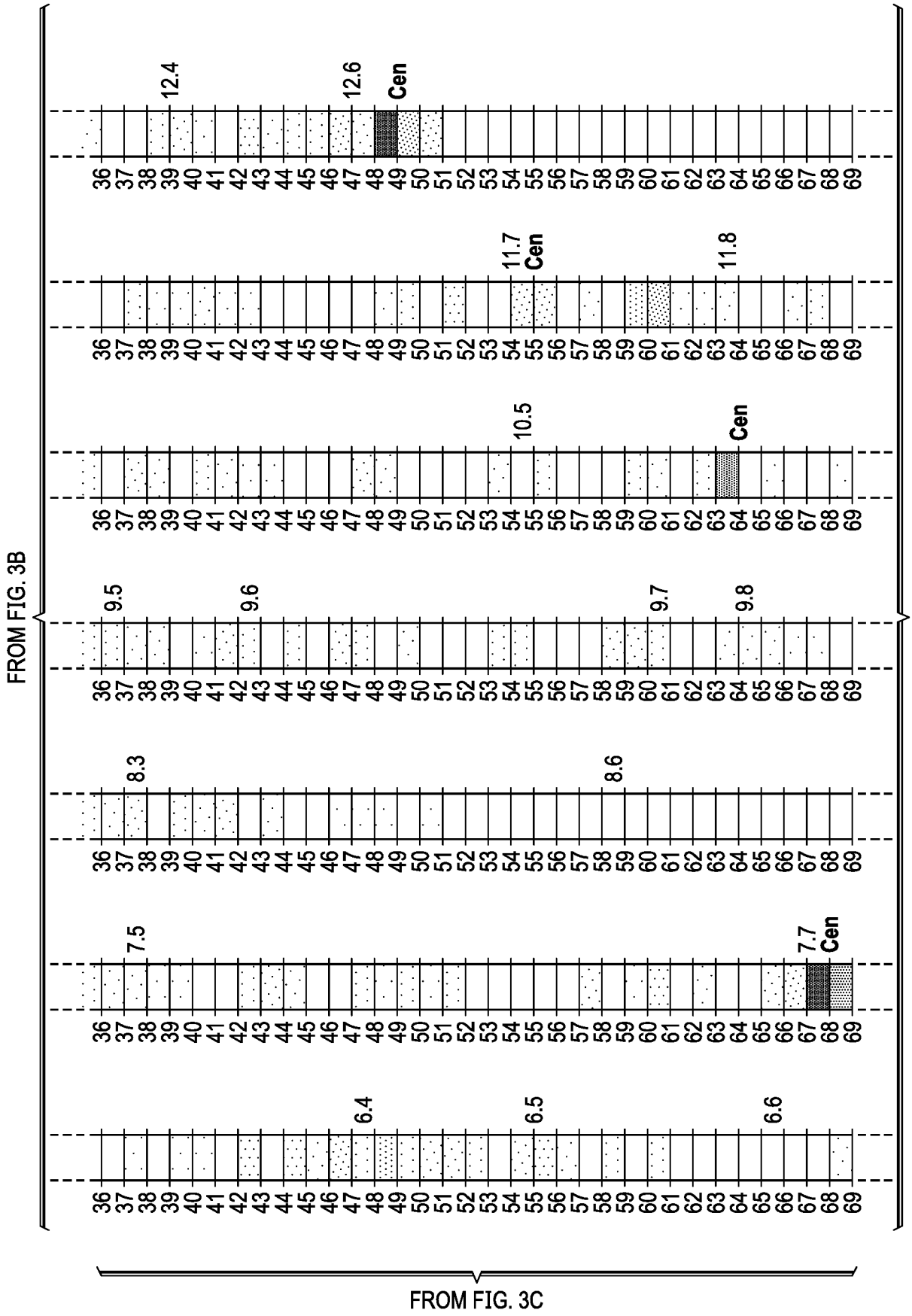


FIG. 3D

REPLACEMENT
SHEET

FIG. 3E

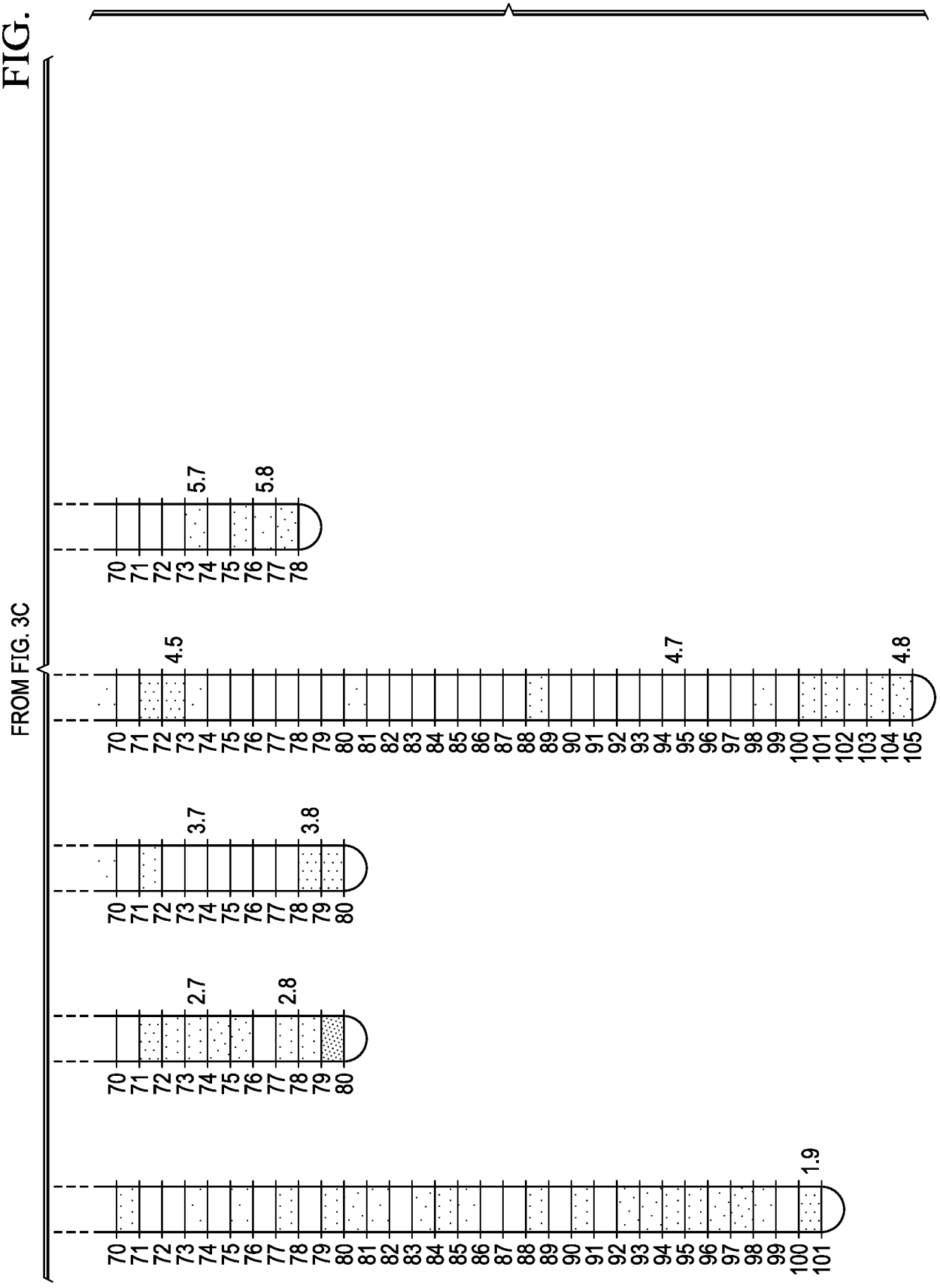
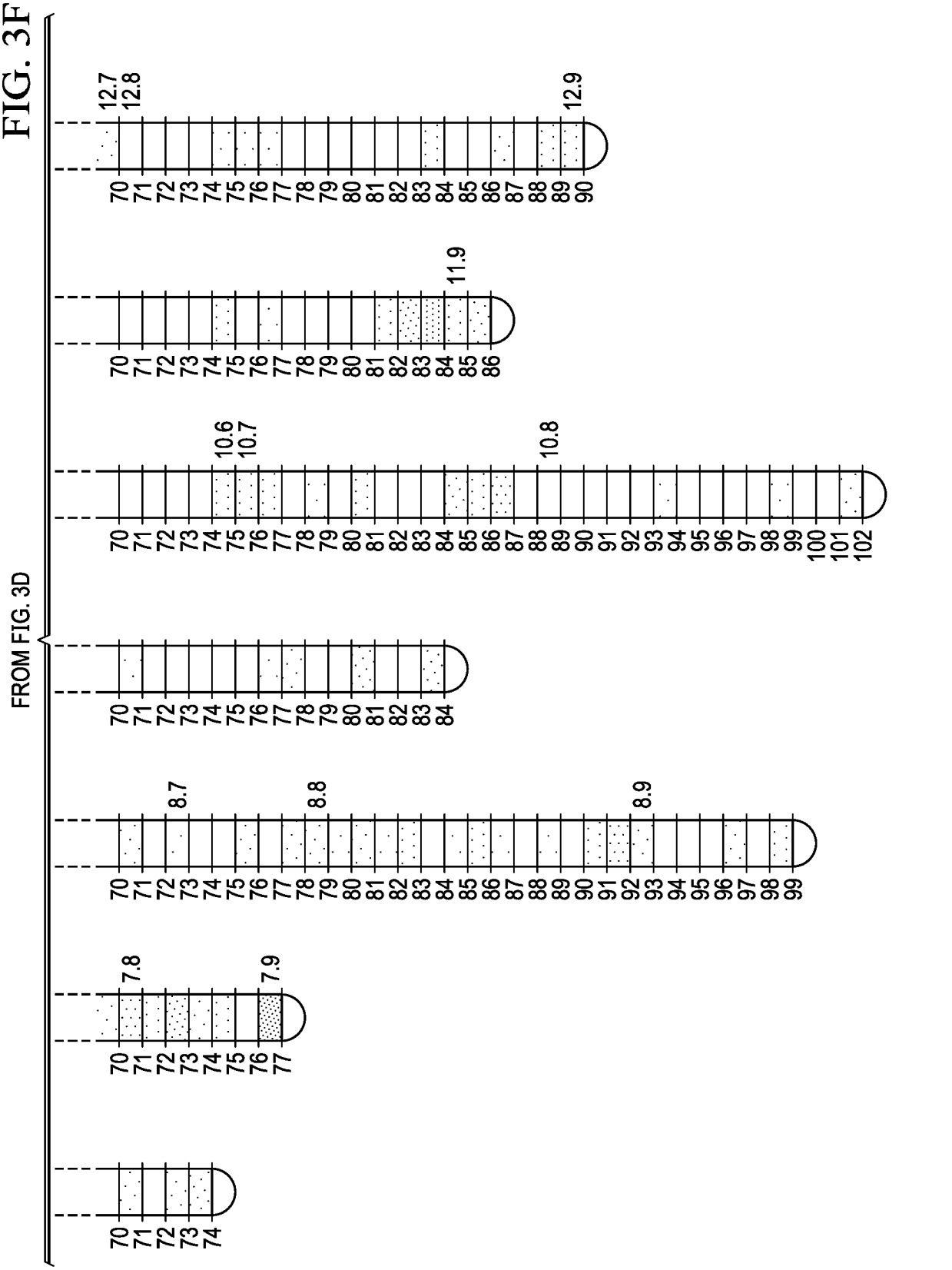


FIG. 3F



TO FIG. 4B

SNP	07-1004-01	IVP97-079-9	IVPAA-096-18	SH83-92-488	C	E	SH83-92-488
PotSNP072	A:A	A:A	G:A	G:A	G:A	A:A	G:A
PotSNP834	C:C	T:C	T:T	T:C	T:C	C:C	T:C
PotSNP645	T:T	X	T:T	T:G	X	G:G	T:G
PotSNP1034	A:A	G:A	G:A	A:A	G:G	G:A	A:A
PotSNP987	C:C	C:A	C:A	X	X	C:A	C:A
PotSNP185	T:T	C:C	C:C	C:C	C:C	C:C	C:C
PotSNP392	T:T	T:A	T:A	T:T	A:A	T:A	T:T
PotSNP165	G:G	G:G	G:A	G:A	G:G	G:G	G:A
PotSNP1139	G:G	G:G	G:G	G:G	G:G	G:G	G:G
PotSNP038	C:C	C:T	C:T	C:T	C:T	C:T	C:T
PotSNP910	A:A	A:A	T:A	T:A	A:A	T:A	T:A
PotSNP106	G:G	G:G	G:G	G:G	G:G	G:T	G:G
PotSNP893	A:A	A:A	A:G	A:A	A:A	A:G	A:A
PotSNP401	A:A	G:A	G:G	G:G	G:A	G:A	G:G
PotSNP220	C:C	C:T	C:C	C:C	C:C	C:C	C:C
PotSNP567	A:A	A:C	A:A	A:A	A:C	A:A	A:A
PotSNP947	T:T	T:G	T:G	T:T	T:T	T:T	T:T
PotSNP055	G:G	G:G	G:A	G:A	G:A	A:A	G:A
PotSNP985	G:G	A:A	A:A	A:A	G:G	A:G	A:A
PotSNP1083	T:T	T:T	C:T	T:T	C:T	C:T	T:T
PotSNP423	G:G	G:G	G:G	G:G	A:G	G:G	G:G
PotSNP1057	C:C	C:C	C:C	A:C	C:C	C:C	A:C
PotSNP068	C:C	C:C	C:C	C:T	C:T	C:T	C:T
PotSNP652	C:C	T:T	C:C	T:C	T:C	C:C	T:C
PotSNP796	C:C	C:A	C:A	C:A	C:A	C:A	C:A
PotSNP178	T:T	T:T	T:T	T:T	G:T	T:T	T:T
PotSNP1072	T:T	T:T	T:T	T:T	G:T	T:T	T:T
PotSNP960	C:C	C:C	C:T	C:T	T:T	C:T	C:T

TO FIG. 4D

FIG. 4A

REPLACEMENT SHEET

FROM FIG. 4A

TO FIG. 4C

RH89-039-16	3778-16	IVP92-057-3	IVP98-082-14	IPV02-089-5	IVP05-113-1	IVP05-122-24
A:A	G:A	G:A	A:A	A:A	G:G	G:A
T:C	C:C	T:T	T:C	T:C	X	T:T
G:G	T:G	G:G	G:G	T:G	T:G	G:G
G:A	G:G	G:A	A:A	A:A	A:A	A:A
C:C	C:C	C:C	C:A	C:A	C:C	X
C:T	C:T	C:C	C:C	C:C	C:C	C:C
T:A	A:A	T:A	T:A	T:T	T:A	T:A
G:G	X	G:G	G:G	G:G	G:A	G:G
G:A	G:G	G:G	G:G	G:G	G:G	G:G
C:T	C:T	T:T	C:T	T:T	C:C	C:C
T:A	A:A	T:A	T:A	T:T	T:A	T:A
G:T	G:G	G:T	G:T	G:G	G:T	T:T
A:G	A:G	A:G	A:A	A:A	G:G	A:G
G:A	G:G	G:A	G:G	G:G	A:A	G:G
C:T	C:C	C:C	C:C	C:C	C:T	C:C
A:C	A:C	A:A	A:A	A:A	A:A	A:C
T:G	T:G	T:T	T:T	T:T	T:G	T:G
G:G	G:A	G:A	G:A	G:G	G:G	G:A
A:G	A:G	A:G	G:G	X	A:G	G:G
C:T	T:T	C:T	C:T	T:T	T:T	C:T
A:G	A:G	G:G	G:G	G:G	A:G	A:G
C:C	A:A	C:C	A:C	C:C	A:C	A:C
C:C	C:T	T:T	C:C	C:T	C:T	C:C
T:C	T:C	C:C	T:C	T:C	T:C	T:C
C:A	A:A	C:A	C:A	C:C	C:A	C:C
G:T	T:T	T:T	T:T	T:T	T:T	G:T
G:T	T:T	G:T	G:G	X	T:T	G:T
C:T	C:T	C:T	T:T	T:T	C:C	C:T

TO FIG. 4E

FIG. 4B

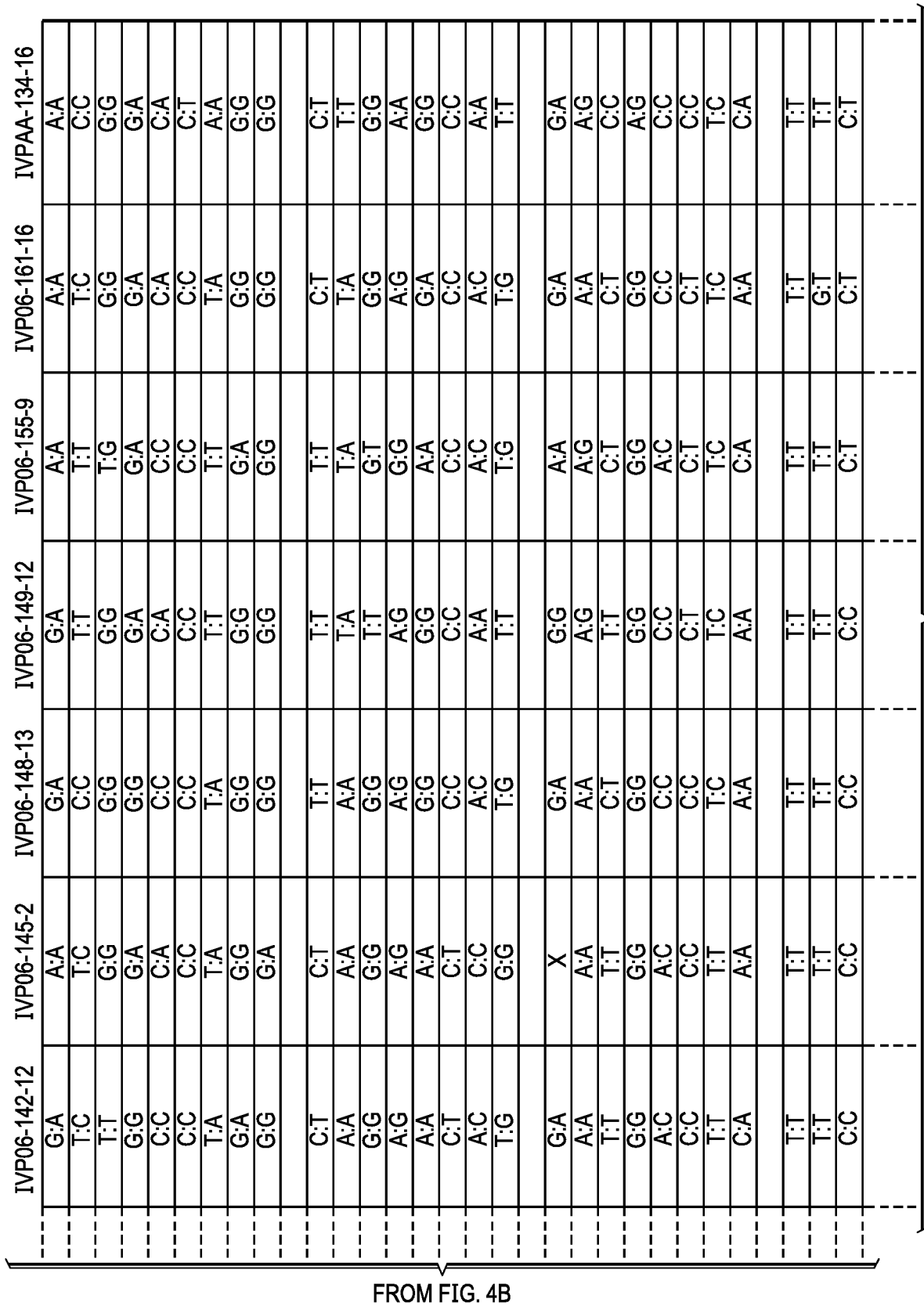

IVP06-142-12
IVP06-145-2
IVP06-148-13
IVP06-149-12
IVP06-155-9
IVP06-161-16
IVPAA-134-16
FROM FIG. 4B
TO FIG. 4F

FIG. 4C

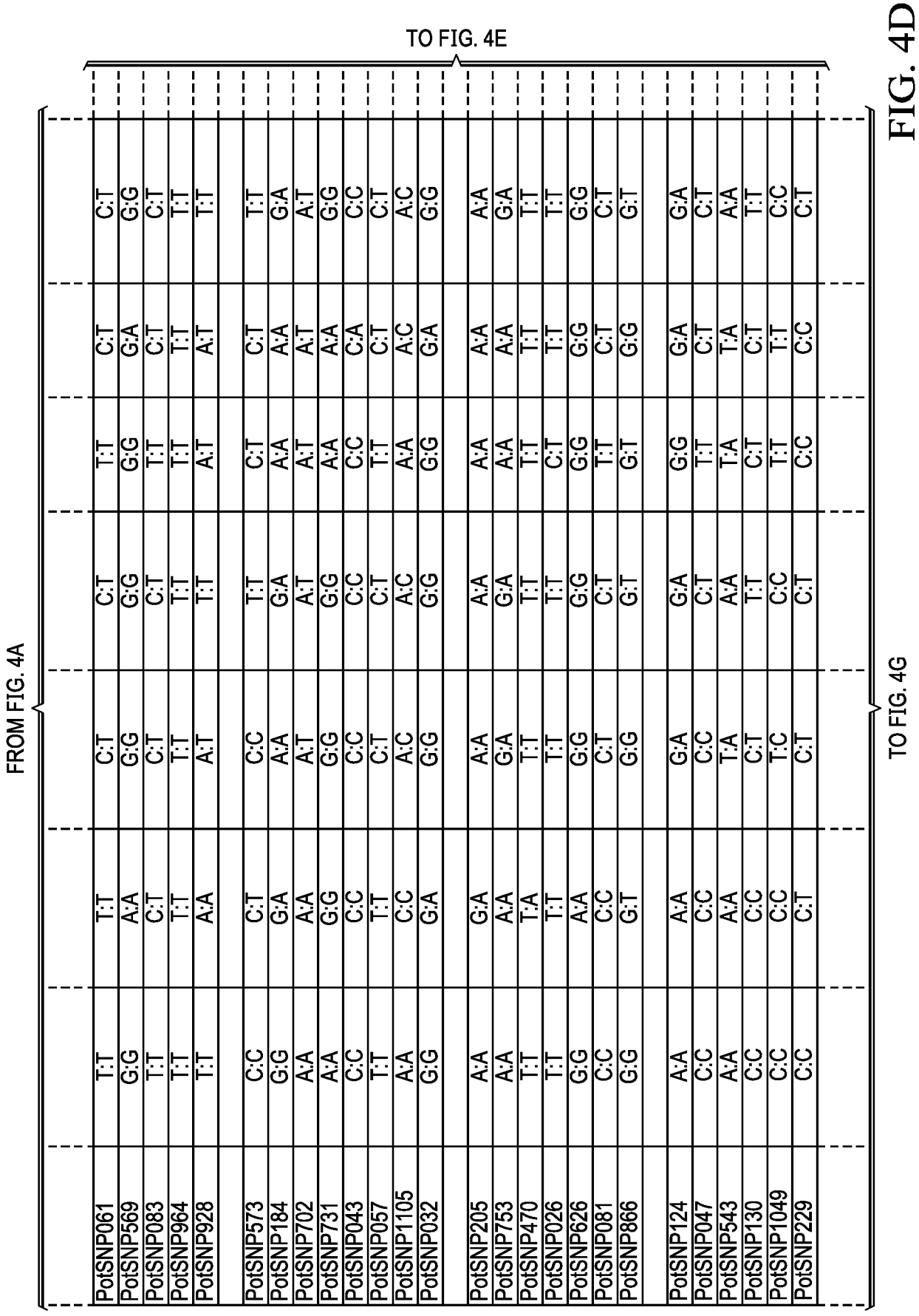

TO FIG. 4E
FROM FIG. 4A
TO FIG. 4G
PotSNP061 T:T T:T C:T C:T T:T C:T C:T
PotSNP569 G:G A:A G:G G:G G:G G:A G:G
PotSNP083 T:T C:T C:T C:T T:T C:T C:T
PotSNP964 T:T T:T T:T T:T T:T T:T T:T
PotSNP928 T:T A:A A:T T:T A:T A:T T:T
PotSNP573 C:C C:T C:C T:T C:T C:T T:T
PotSNP184 G:G G:A A:A G:A A:A A:A G:A
PotSNP702 A:A A:A A:T A:T A:T A:T A:T
PotSNP731 A:A G:G G:G G:G A:A A:A G:G
PotSNP043 C:C C:C C:C C:C C:C C:A C:C
PotSNP057 T:T T:T C:T C:T T:T C:T C:T
PotSNP1105 A:A C:C A:C A:C A:A A:C A:C
PotSNP032 G:G G:A G:G G:G G:G G:A G:G
PotSNP205 A:A G:A A:A A:A A:A A:A A:A
PotSNP753 A:A A:A G:A G:A A:A A:A G:A
PotSNP470 T:T T:A T:T T:T T:T T:T T:T
PotSNP026 T:T T:T T:T T:T C:T T:T T:T
PotSNP626 G:G A:A G:G G:G G:G G:G G:G
PotSNP081 C:C C:C C:T C:T T:T C:T C:T
PotSNP866 G:G G:T G:G G:T G:T G:G G:T
PotSNP124 A:A A:A G:A G:A G:G G:A G:A
PotSNP047 C:C C:C C:C C:T T:T C:T C:T
PotSNP543 A:A A:A T:A A:A T:A T:A A:A
PotSNP130 C:C C:C C:T T:T C:T C:T T:T
PotSNP1049 C:C C:C T:C C:C T:T T:T C:C
PotSNP229 C:C C:T C:T C:T C:C C:C C:T

FIG. 4D

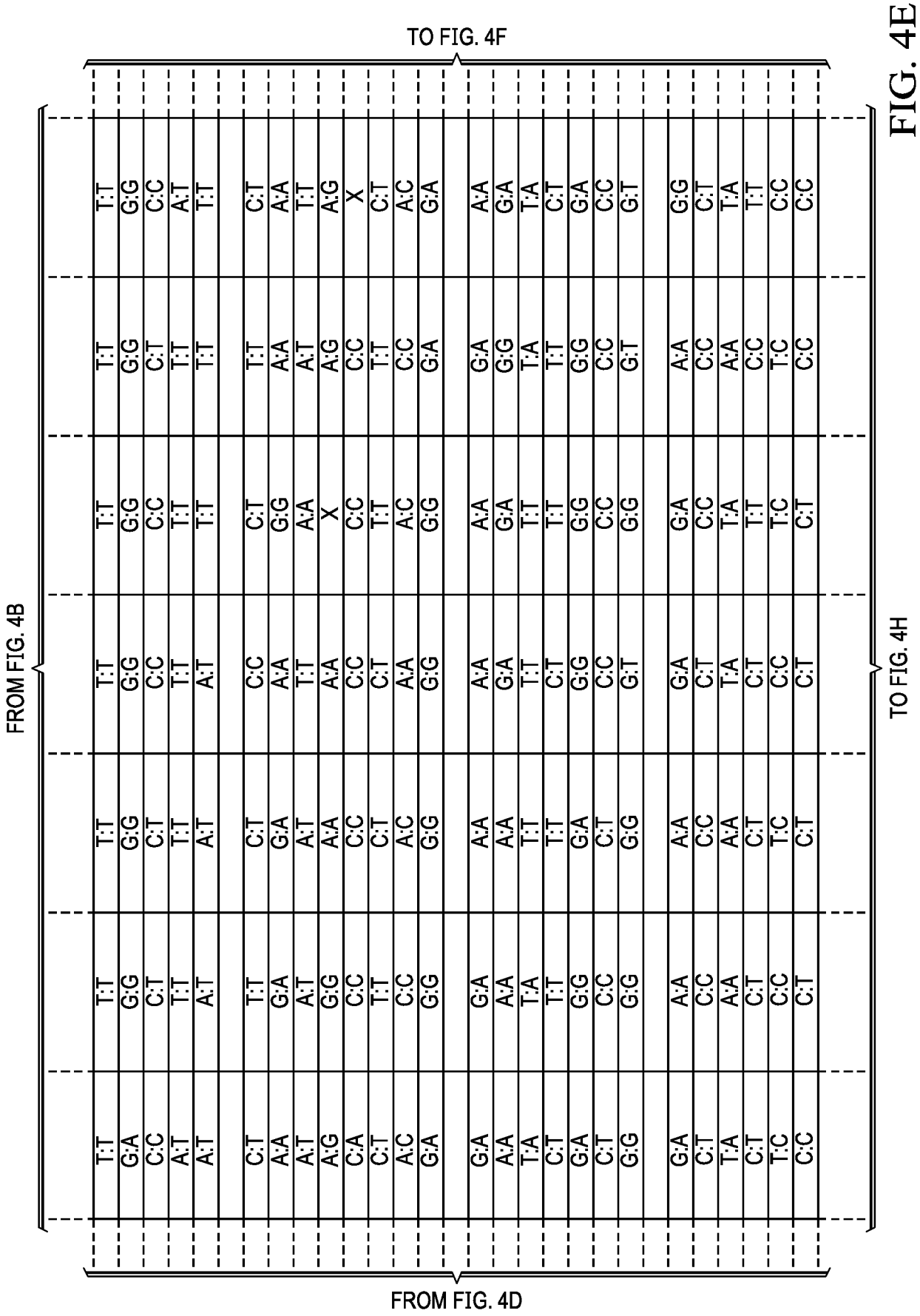


FIG. 4E

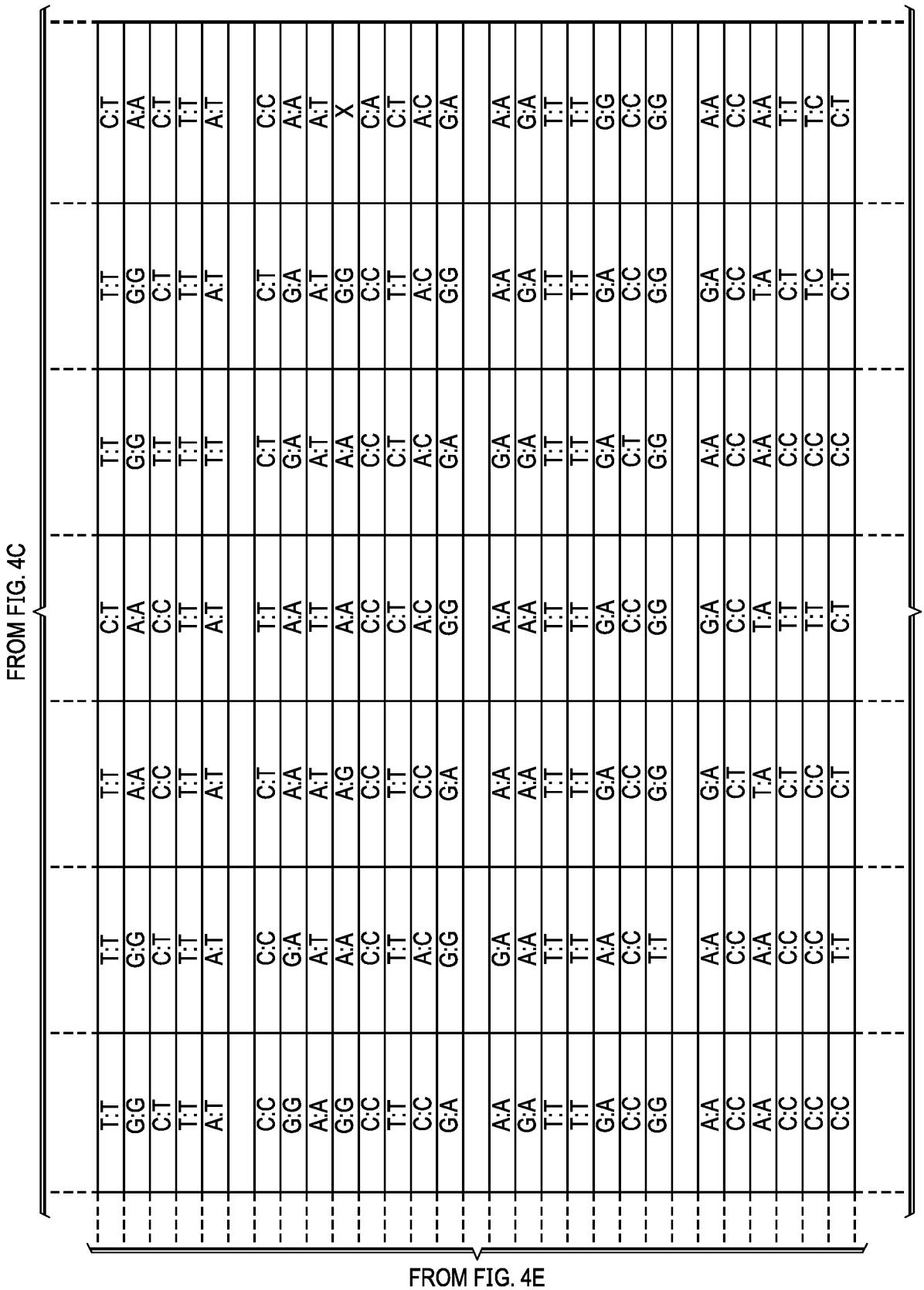


FIG. 4F

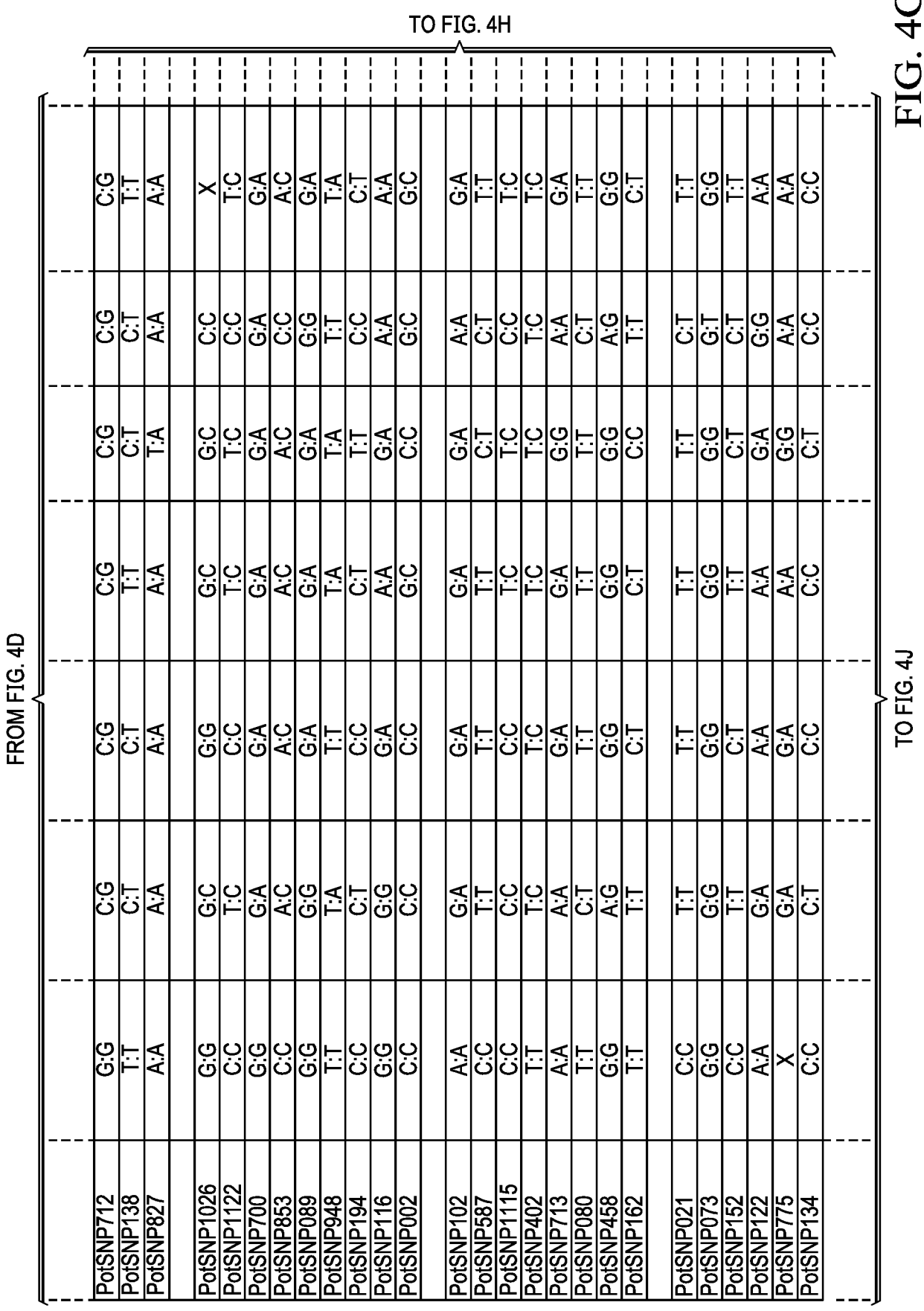
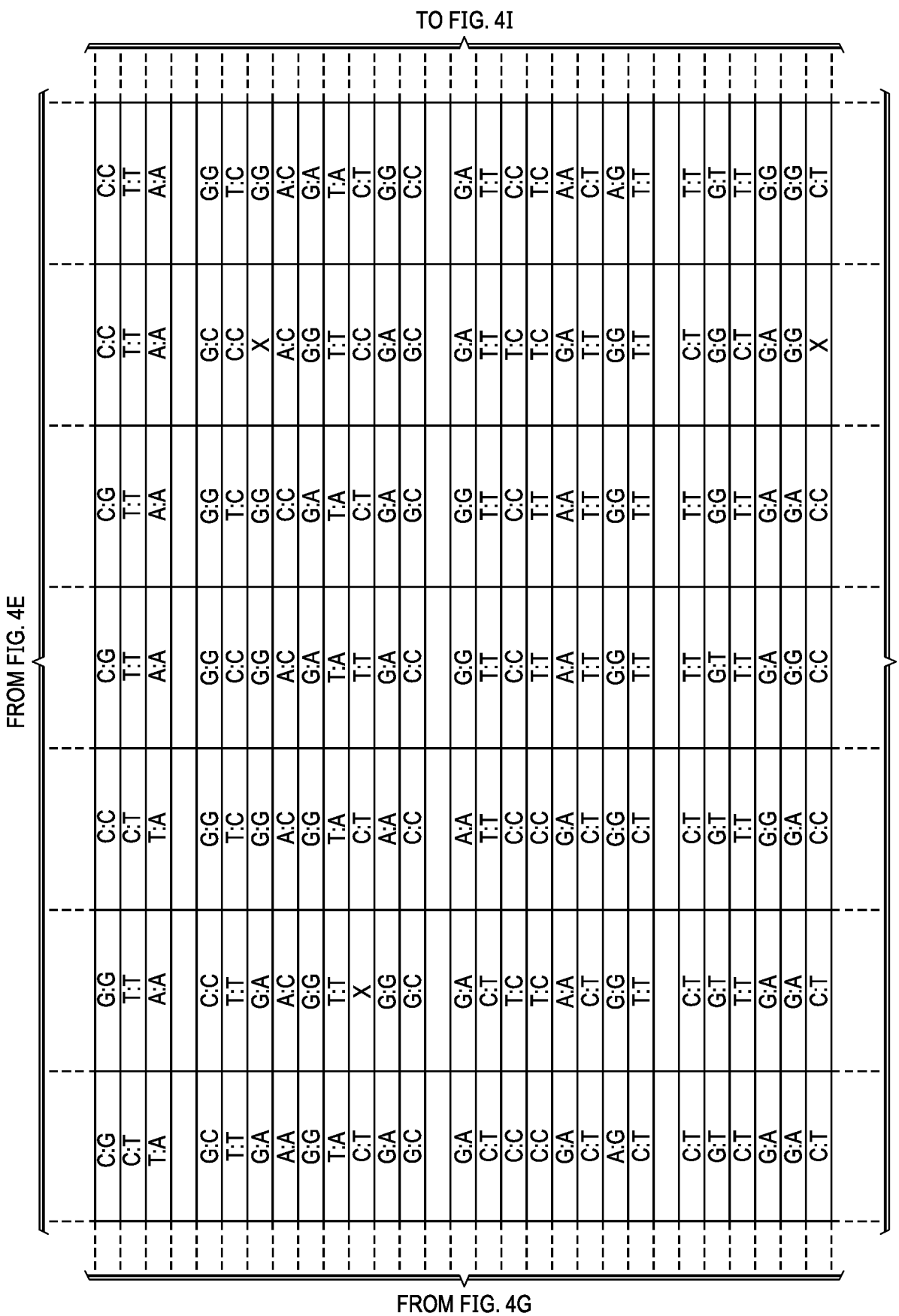

TO FIG. 4H
FROM FIG. 4D
TO FIG. 4J
PotSNP712 G:G C:G C:G C:G C:G C:G C:G
PotSNP138 T:T C:T T:T C:T C:T C:T T:T
PotSNP827 A:A A:A A:A T:A A:A A:A A:A
PotSNP1026 G:G G:C G:G G:C G:C C:C X
PotSNP1122 C:C T:C C:C T:C T:C C:C T:C
PotSNP700 G:G G:A G:A G:A G:A G:A G:A
PotSNP853 C:C A:C A:C A:C A:C C:C A:C
PotSNP089 G:G G:G G:A G:A G:A G:G G:A
PotSNP948 T:T T:A T:T T:A T:A T:T T:A
PotSNP194 C:C C:T C:C C:T T:T C:C C:T
PotSNP116 G:G G:G G:A A:A G:A A:A A:A
PotSNP002 C:C C:C C:C G:C C:C G:C G:C
PotSNP102 A:A G:A G:A G:A G:A A:A G:A
PotSNP587 C:C T:T T:T T:T C:T C:T T:T
PotSNP1115 C:C C:C C:C T:C T:C C:C T:C
PotSNP402 T:T T:C T:C T:C T:C T:C T:C
PotSNP713 A:A A:A G:A G:A G:G A:A G:A
PotSNP080 T:T C:T T:T T:T T:T C:T T:T
PotSNP458 G:G A:G G:G G:G G:G A:G G:G
PotSNP162 T:T T:T C:T C:T C:C T:T C:T
PotSNP021 C:C T:T T:T T:T T:T C:T T:T
PotSNP073 G:G G:G G:G G:G G:G G:T G:G
PotSNP152 C:C T:T C:T T:T C:T C:T T:T
PotSNP122 A:A G:A A:A A:A G:A G:G A:A
PotSNP775 X G:A G:A A:A G:G A:A A:A
PotSNP134 C:C C:T C:C C:C C:T C:C C:C

FIG. 4G

REPLACEMENT SHEET

FIG. 4H



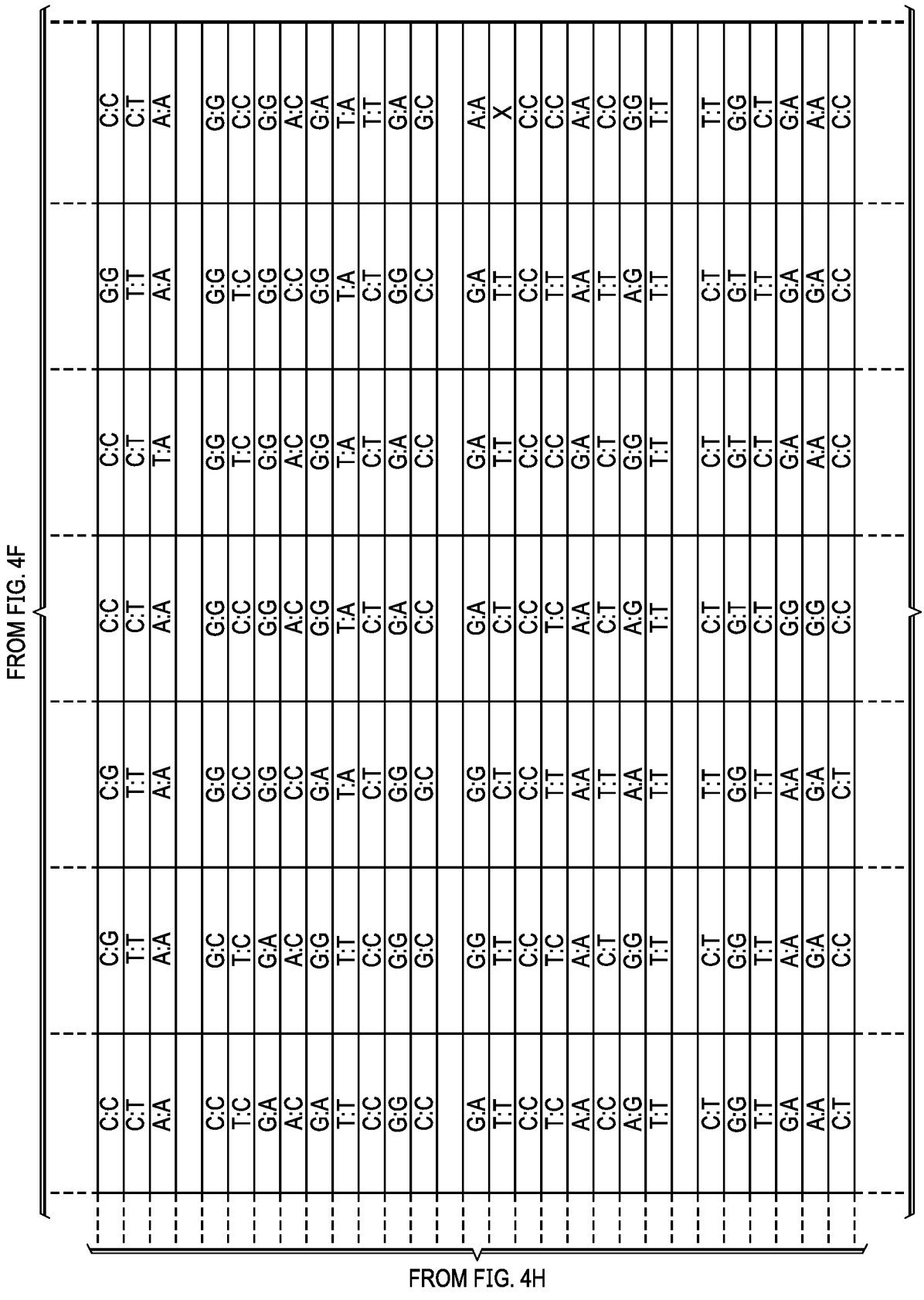
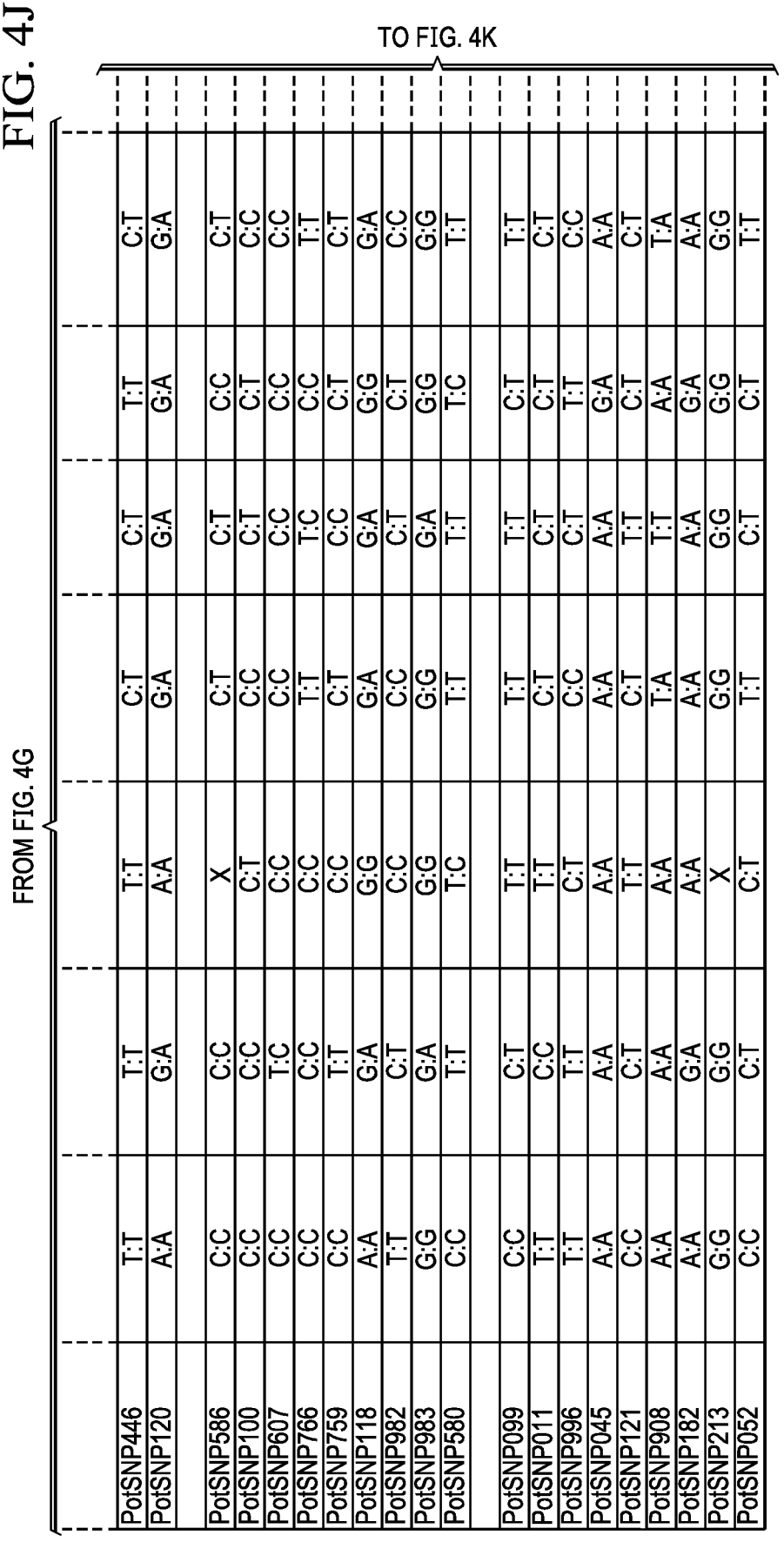


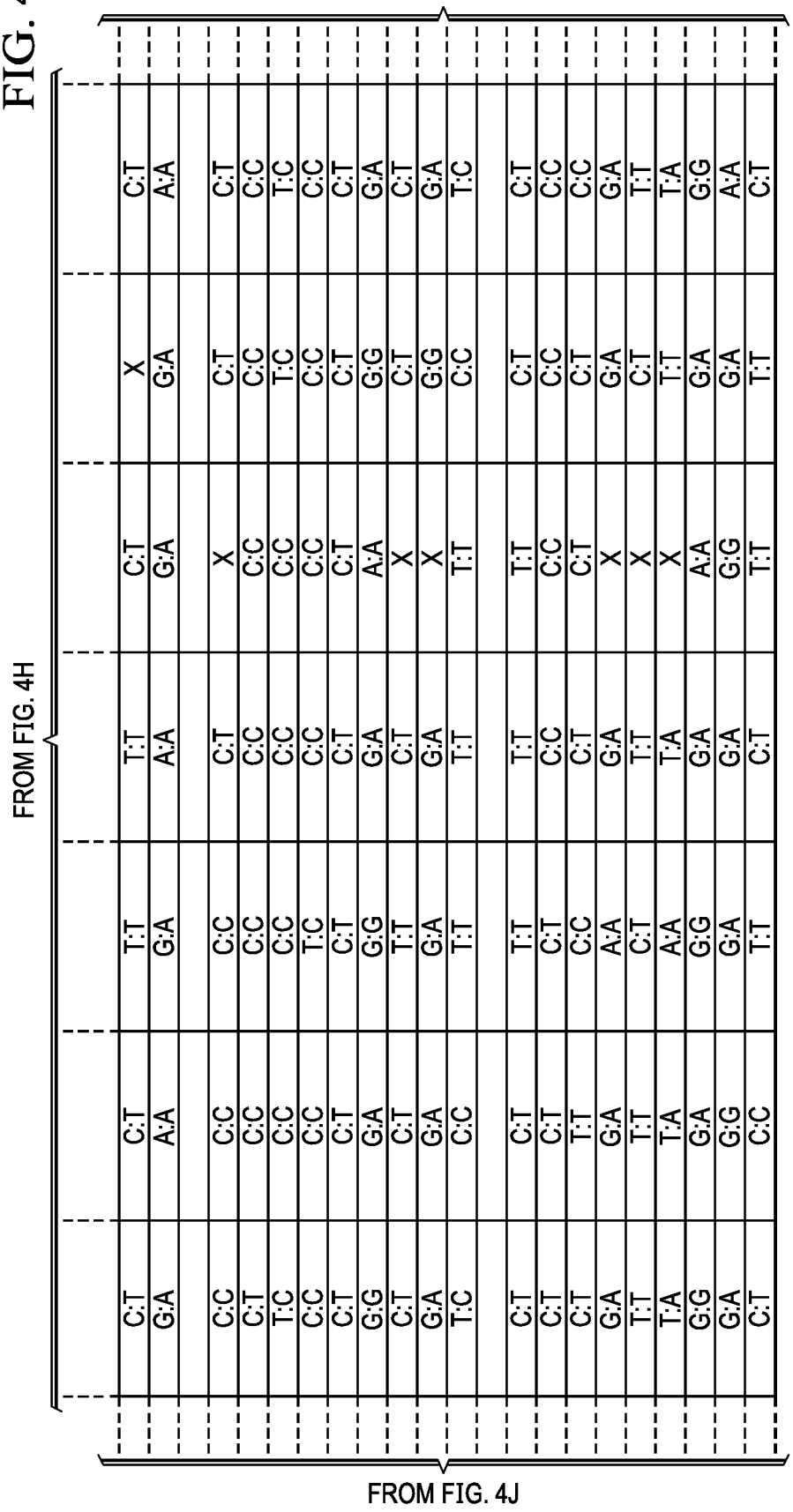
FIG. 4I

FIG. 4J



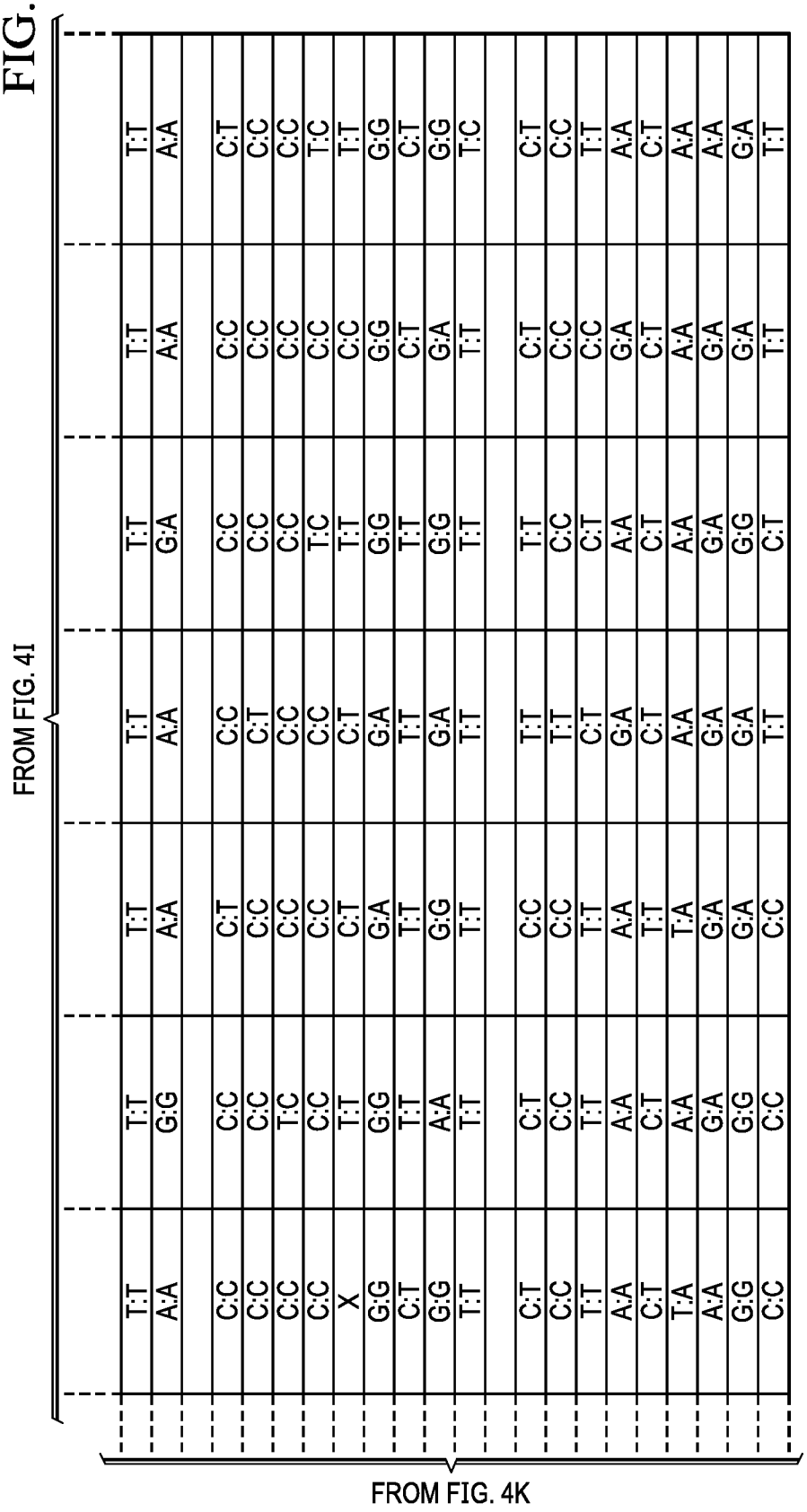
PotSNP446	T:T	T:T	T:T	C:T	C:T	T:T	C:T		
PotSNP120	A:A	G:A	A:A	G:A	G:A	G:A	G:A		
PotSNP586	C:C	C:C	X	C:T	C:T	C:C	C:T		
PotSNP100	C:C	C:C	C:T	C:C	C:T	C:T	C:C		
PotSNP607	C:C	T:C	C:C	C:C	C:C	C:C	C:C		
PotSNP766	C:C	C:C	C:C	T:T	T:C	C:C	T:T		
PotSNP759	C:C	T:T	C:C	C:T	C:C	C:T	C:T		
PotSNP118	A:A	G:A	G:G	G:A	G:A	G:G	G:A		
PotSNP982	T:T	C:T	C:C	C:C	C:T	C:T	C:C		
PotSNP983	G:G	G:A	G:G	G:G	G:A	G:G	G:G		
PotSNP580	C:C	T:T	T:C	T:T	T:T	T:C	T:T		
PotSNP099	C:C	C:T	T:T	T:T	T:T	C:T	T:T		
PotSNP011	T:T	C:C	T:T	C:T	C:T	C:T	C:T		
PotSNP996	T:T	T:T	C:T	C:C	C:T	T:T	C:C		
PotSNP045	A:A	A:A	A:A	A:A	A:A	G:A	A:A		
PotSNP121	C:C	C:T	T:T	C:T	T:T	C:T	C:T		
PotSNP908	A:A	A:A	A:A	T:A	T:T	A:A	T:A		
PotSNP182	A:A	G:A	A:A	A:A	A:A	G:A	A:A		
PotSNP213	G:G	G:G	X	G:G	G:G	G:G	G:G		
PotSNP052	C:C	C:T	C:T	T:T	C:T	C:T	T:T		

FIG. 4K



C:T	C:T	T:T	C:T	X	C:T	C:T
G:A	A:A	A:A	G:A	G:A	G:A	A:A
C:C	C:C	C:T	X	C:T	C:T	C:T
C:T	C:C	C:C	C:C	C:C	C:C	C:C
T:C	C:C	C:C	C:C	T:C	C:C	T:C
C:C	C:C	C:C	C:C	C:C	C:C	C:C
C:T	C:T	C:T	C:T	C:T	C:T	C:T
G:G	G:A	G:A	A:A	G:G	G:A	G:A
C:T	C:T	C:T	X	C:T	C:T	C:T
G:A	C:T	G:A	X	G:G	G:A	G:A
T:C	C:C	T:T	T:T	C:C	T:T	T:C
C:T	C:T	T:T	T:T	C:T	C:T	C:T
C:T	C:T	C:C	C:C	C:C	C:C	C:C
C:T	T:T	C:C	C:T	C:T	C:T	C:C
G:A	G:A	A:A	G:A	X	G:A	G:A
T:T	C:T	C:T	T:T	X	C:T	T:T
T:A	T:A	A:A	T:A	X	T:T	T:A
G:G	G:A	G:G	G:A	A:A	G:A	G:G
G:A	G:G	G:A	G:A	G:G	G:A	A:A
C:T	C:C	T:T	C:T	T:T	T:T	C:T

FIG. 4L



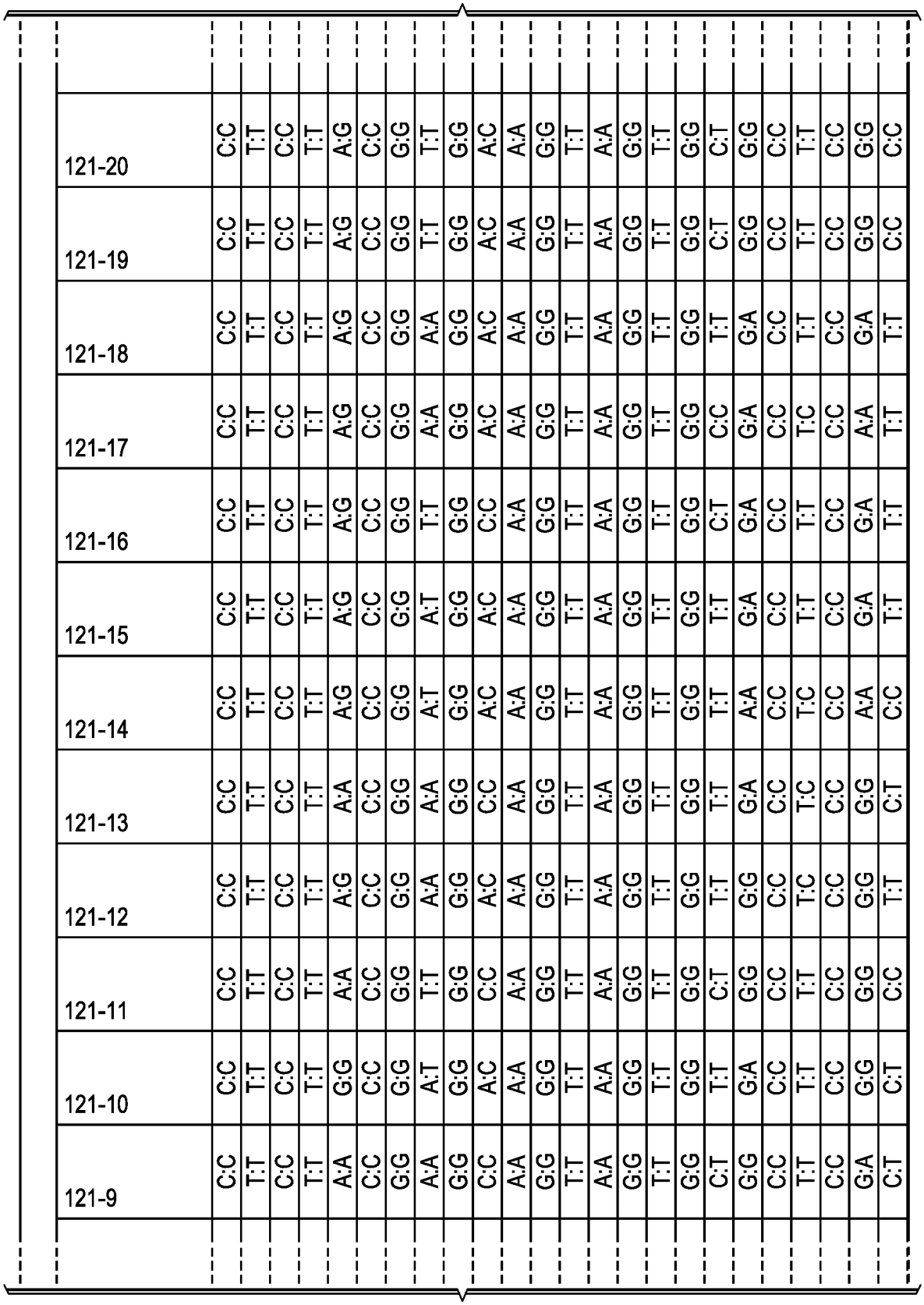
TO FIG. 5A-2

	H	D1	F1		F3 population no 121								
SNP	07-1004-1	IVP97-079-9	IVP08-206-32		121-1	121-2	121-3	121-4	121-5	121-6	121-7	121-8	
PotSNP834	C:C	T:C	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	
PotSNP392	T:T	T:A	T:A		T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	
PotSNP038	C:C	C:T	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	
PotSNP947	T:T	T:G	T:T		T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	
PotSNP985	G:G	A:A	A:G		A:G	A:G	A:G	A:G	A:G	A:G	A:G	A:G	
PotSNP796	C:C	C:A	C:A		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	
PotSNP569	G:G	A:A	G:A		G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	
PotSNP928	T:T	A:A	A:T		A:T	A:T	A:A	A:A	T:T	A:T	A:A	A:A	
PotSNP184	G:G	G:A	G:A		G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	
PotSNP1105	A:A	C:C	A:C		A:C	C:C	A:C	A:A	C:C	?	A:A	C:C	
PotSNP205	A:A	G:A	A:A		A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	
PotSNP626	G:G	A:A	G:A		G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	
PotSNP229	C:C	C:T	C:T		T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	
PotSNP102	A:A	G:A	G:A		A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	
PotSNP458	G:G	G:A	A:G		G:G	G:G	G:G	?	G:G	G:G	G:G	G:G	
PotSNP138	T:T	C:T	T:T		T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	
PotSNP1026	G:G	G:C	G:G		G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	
PotSNP152	C:C	C:T	C:T		C:T	T:T	C:T	T:T	T:T	C:T	C:C	C:T	
PotSNP120	A:A	G:A	G:A		G:A	G:G	G:A	A:A	G:A	G:G	G:A	G:G	
PotSNP607	C:C	T:C	T:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	
PotSNP580	C:C	T:T	T:C		C:C	T:C	T:C	T:C	T:C	T:C	C:C	T:C	
PotSNP099	C:C	C:T	C:T		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	
PotSNP182	A:A	G:A	G:A		G:A	G:G	G:A	A:A	G:A	G:A	G:A	G:G	
PotSNP052	C:C	C:T	C:C		C:T	C:T	T:T	T:T	T:T	C:T	C:C	C:T	

FIG. 5A-1

FIG. 5A-2

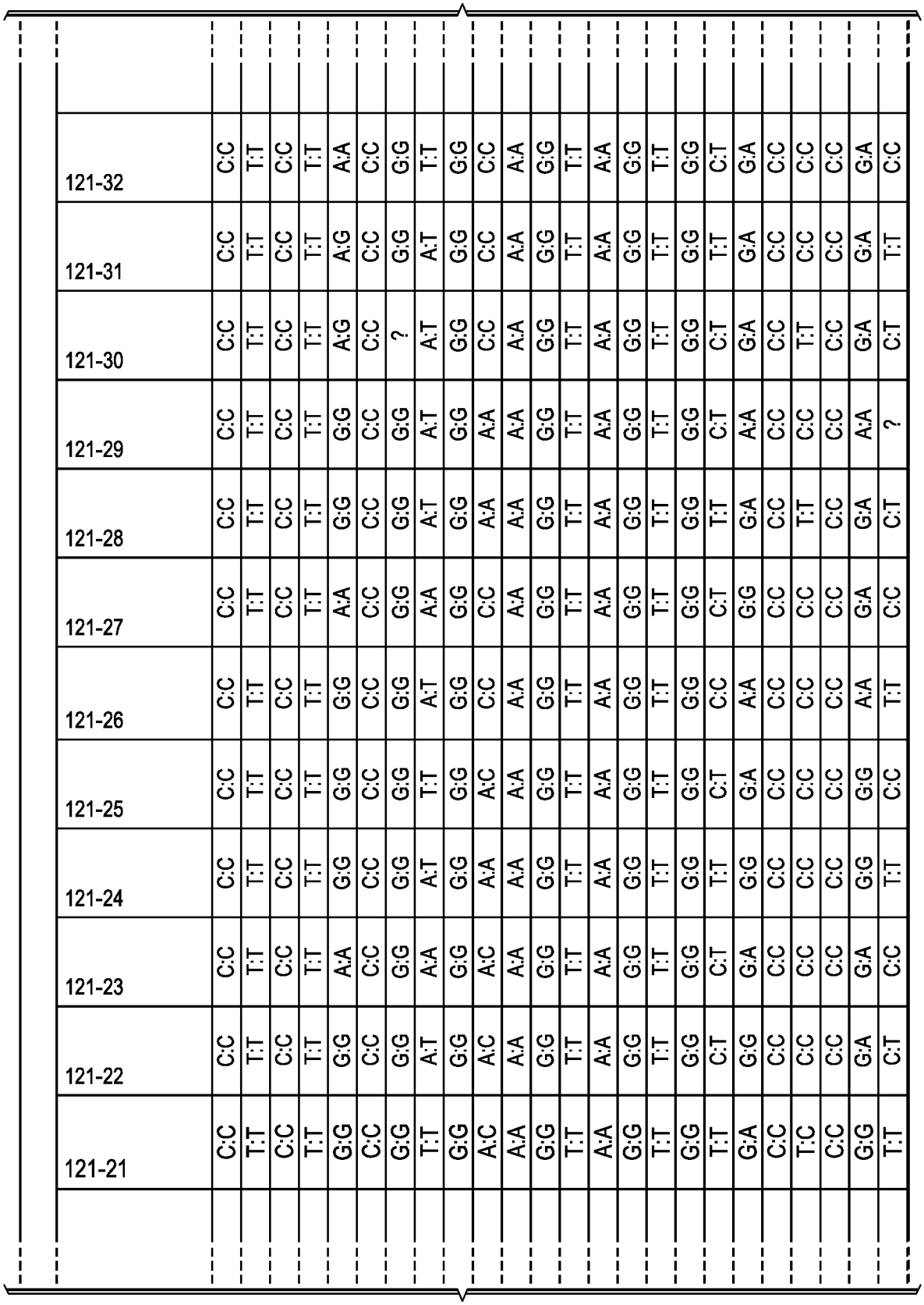
TO FIG. 5A-3



	121-20	121-19	121-18	121-17	121-16	121-15	121-14	121-13	121-12	121-11	121-10	121-9	
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	
	A:G	A:G	A:G	A:G	A:G	A:G	A:G	A:A	A:G	A:A	G:G	A:A	
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	
	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	
	T:T	T:T	A:A	A:A	T:T	A:T	A:T	A:A	A:A	T:T	A:T	A:A	
	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	
	A:C	A:C	A:C	A:C	C:C	A:C	A:C	C:C	A:C	C:C	A:C	C:C	
	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	
	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	
	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	
	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	
	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	
	C:T	C:T	T:T	C:C	C:T	T:T	T:T	T:T	T:T	C:T	T:T	C:T	
	G:G	G:G	G:A	G:A	G:A	G:A	G:A	G:A	G:A	G:G	G:A	G:G	
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	
	T:T	T:T	T:T	T:C	T:T	T:T	T:C	T:C	T:C	T:T	T:C	T:T	
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	
	G:G	G:G	G:A	A:A	G:A	G:A	A:A	G:G	G:G	G:G	G:G	G:A	
	C:C	C:C	T:T	T:T	T:T	T:T	C:C	C:T	T:T	C:C	C:T	C:T	

FROM FIG. 5A-1

TO FIG. 5A-4

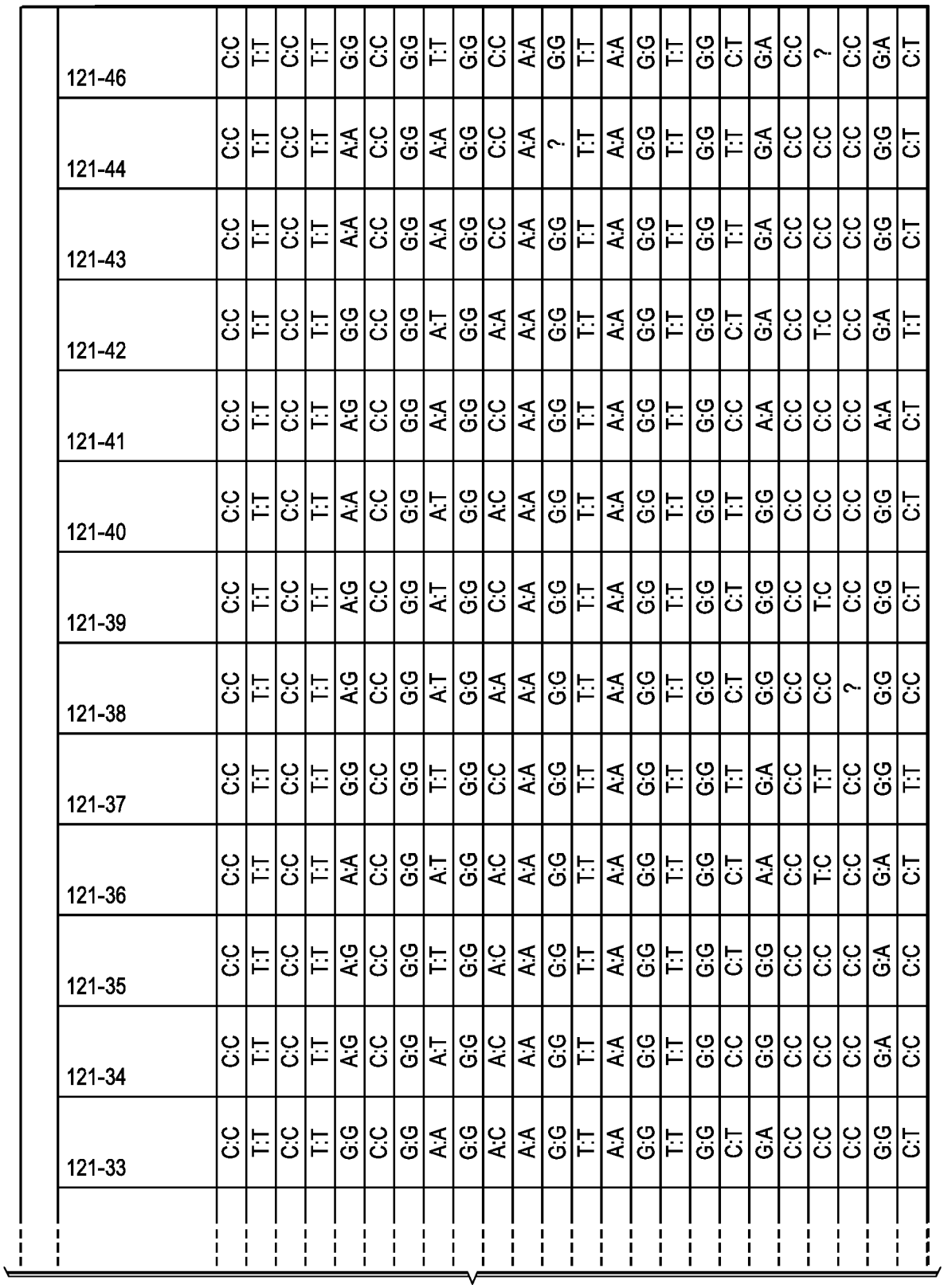


	121-32	121-31	121-30	121-29	121-28	121-27	121-26	121-25	121-24	121-23	121-22	121-21	
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	
	A:A	A:G	A:G	G:G	G:G	A:A	G:G	G:G	G:G	A:A	G:G	G:G	
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	
	G:G	G:G	?	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	
	T:T	A:T	A:T	A:T	A:T	A:A	A:T	T:T	A:T	A:A	A:T	T:T	
	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	
	C:C	C:C	C:C	A:A	A:A	C:C	C:C	A:C	A:A	A:C	A:C	A:C	
	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	
	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	
	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	
	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	
	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	
	C:T	C:T	C:T	C:T	T:T	C:T	C:C	C:T	T:T	C:T	C:T	T:T	
	G:A	G:A	G:A	A:A	G:A	G:G	A:A	G:A	G:G	G:A	G:G	G:G	
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	
	C:C	C:C	T:T	C:C	T:T	C:C	C:C	C:C	C:C	C:C	C:C	T:C	
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	
	G:A	G:A	G:A	A:A	G:A	G:A	A:A	G:G	G:G	G:A	G:A	G:G	
	C:C	T:T	C:T	?	C:T	C:C	T:T	C:C	T:T	C:C	C:T	T:T	

FIG. 5A-3

FROM FIG. 5A-2

FIG. 5A-4

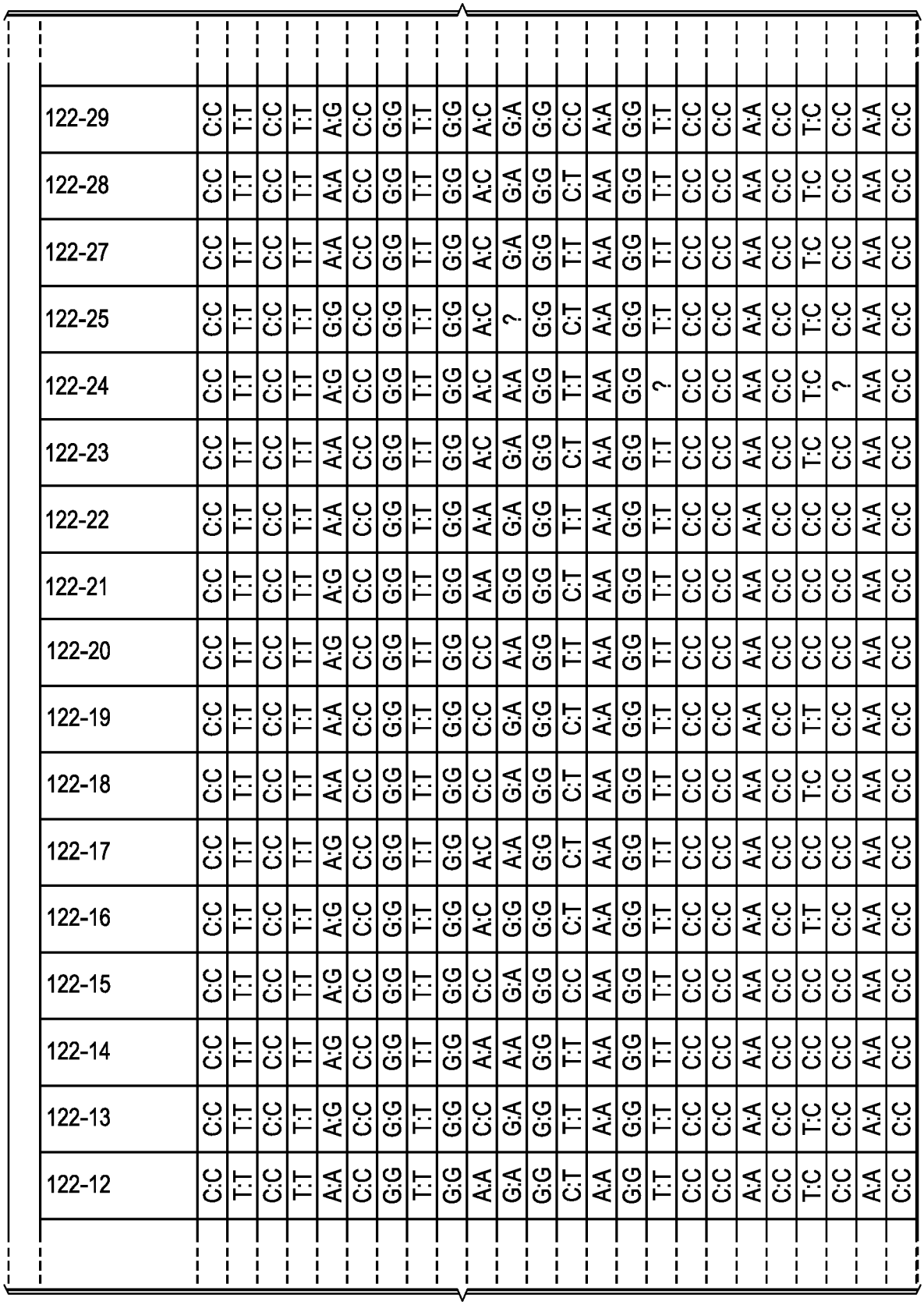

121-46
121-44
121-43
121-42
121-41
121-40
121-39
121-38
121-37
121-36
121-35
121-34
121-33
FROM FIG. 5A-3

TO FIG. 5B-2

	H	D1	F1	F2	F3 population no 122											
SNP	07-1004-1	IVP97-079-9	IVP08-206-32	AGV09-007-09		122-1	122-2	122-3	122-4	122-5	122-6	122-7	122-8	122-9	122-10	122-11
PotSNP834	C:C	T:C	C:C	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
PotSNP392	T:T	T:A	T:A	T:T		T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
PotSNP038	C:C	C:T	C:C	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
PotSNP947	T:T	T:G	T:T	T:T		T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
PotSNP985	G:G	A:A	A:G	A:G		A:A	A:G	A:G	A:G	G:G	A:A	A:G	A:G	A:G	G:G	A:G
PotSNP796	C:C	C:A	C:A	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
PotSNP569	G:G	A:A	G:A	G:G		G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
PotSNP928	T:T	A:A	A:T	T:T		T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
PotSNP184	G:G	G:A	G:A	G:G		G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
PotSNP1105	A:A	C:C	A:C	A:C		A:C	A:C	A:A	A:C	A:C	A:A	A:A	A:A	A:A	A:C	A:C
PotSNP205	A:A	G:A	A:A	G:A		G:A	G:A	A:A	G:A	G:A	G:A	A:A	G:G	A:A	G:A	G:A
PotSNP626	G:G	A:A	G:A	G:G		G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
PotSNP229	C:C	C:T	C:T	C:T		C:T	C:T	T:T	C:T	T:T	T:T	T:T	C:T	C:T	C:T	C:T
PotSNP102	A:A	G:A	G:A	A:A		A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
PotSNP458	G:G	G:A	A:G	G:G		G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
PotSNP138	T:T	C:T	T:T	T:T		T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
PotSNP1026	G:G	G:C	G:G	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
PotSNP152	C:C	C:T	C:T	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
PotSNP120	A:A	G:A	G:A	A:A		A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
PotSNP607	C:C	T:C	T:C	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
PotSNP580	C:C	T:T	T:C	T:C		T:C	T:C	C:C	T:C	T:T	T:C	C:C	T:C	T:C	T:C	T:T
PotSNP099	C:C	C:T	C:T	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
PotSNP182	A:A	G:A	G:A	A:A		A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
PotSNP052	C:C	C:T	C:C	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C

FIG. 5B-1

TO FIG. 5B-3



	122-29	122-28	122-27	122-25	122-24	122-23	122-22	122-21	122-20	122-19	122-18	122-17	122-16	122-15	122-14	122-13	122-12
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	T:T	A:A	A:A	G:G	A:G	A:A	A:A	A:G	A:G	A:A	A:A	A:G	A:G	A:G	A:G	A:G	A:A
	A:G	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	A:C	A:C	A:C	A:C	A:C	A:C	A:C	A:A	C:C	C:C	C:C	A:C	A:C	C:C	A:A	C:C	A:A
	G:A	G:A	G:A	?	A:A	G:A	G:A	G:G	A:A	G:A	G:A	A:A	G:G	A:A	A:A	G:A	G:A
	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	C:T	C:T	T:T	C:T	T:T	C:T	T:T	C:T	T:T	C:T	C:T	C:T	C:T	C:C	T:T	T:T	C:T
	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	T:T	T:T	T:T	T:T	?	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	T:C	T:C	T:C	T:C	T:C	T:C	T:C	C:C	C:C	T:T	T:C	C:C	T:T	C:C	C:C	T:C	T:C
	C:C	C:C	C:C	C:C	?	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C

FROM FIG. 5B-1

FIG. 5B-2

FIG. 5B-3

	122-30	122-31	122-32	122-33	122-34	122-35	122-36	122-37	122-38	122-39	122-40	122-41	122-42	122-43
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	A:A	A:A	A:G	G:G	A:G	G:G	A:G	A:A	A:G	A:G	G:G	A:G	A:A	A:G
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	A:C	A:C	A:C	C:C	A:C	C:C	A:C	A:A	A:A	A:C	C:C	A:C	C:C	A:A
	G:A	G:A	A:A	A:A	G:A	G:A	A:A	G:A	A:A	G:A	G:A	G:A	G:A	G:A
	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	T:T	C:C	T:T	C:C	?	C:C	C:T	C:C	C:C	C:T	C:T	C:C	C:T	T:T
	A:A	A:A	A:A	A:A	?	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	T:T	C:C	T:T	C:C	?	T:C	T:C	T:C	C:C	T:T	T:C	T:T	T:T	T:C
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C

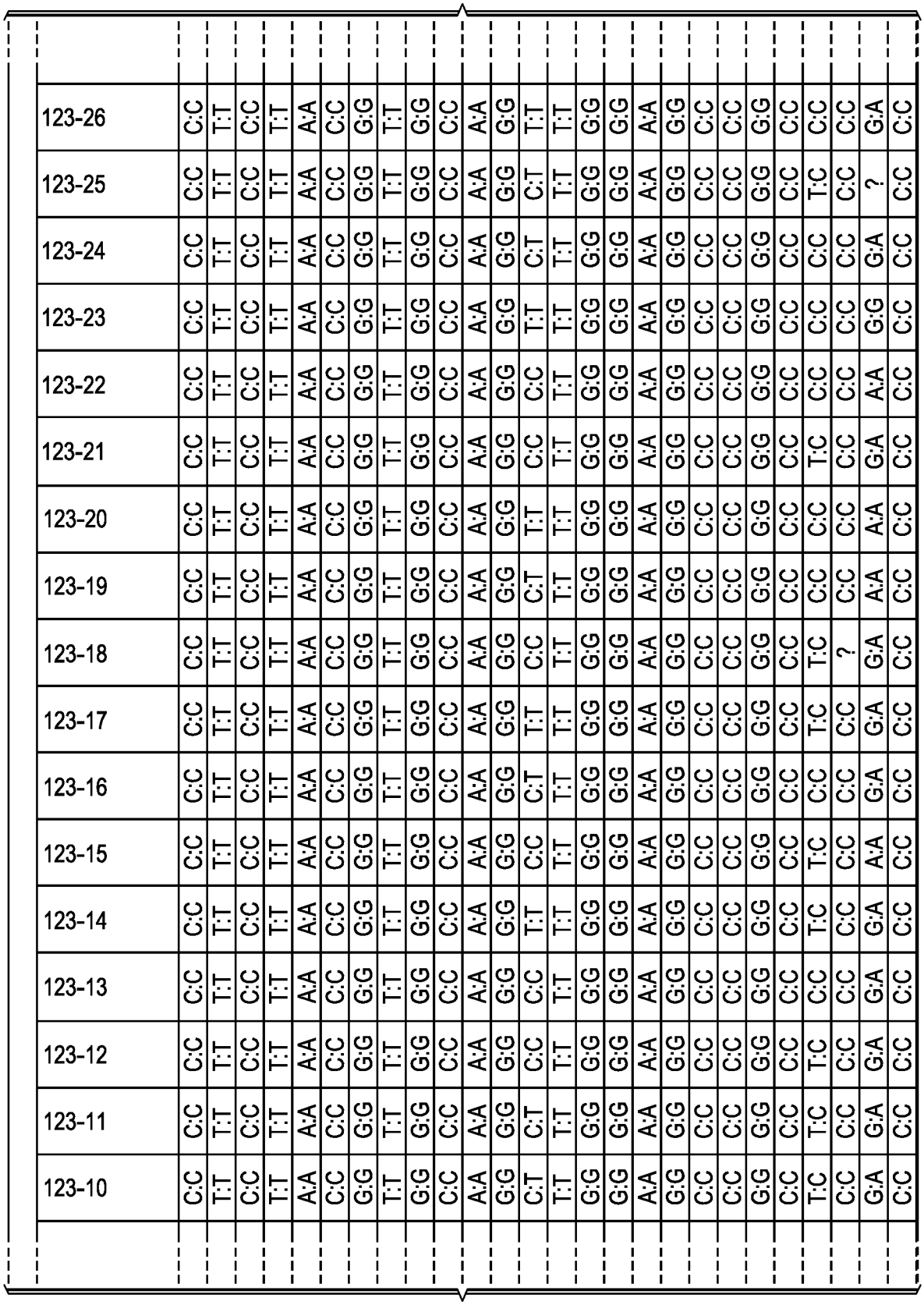
FROM FIG. 5B-2

FIG. 5C-1

TO FIG. 5C-2

					F3 population 123								
	SNP	H	D1	F1	F2	123-1	123-2	123-3	123-4	123-5	123-6	123-7	123-8	123-9
		07-1004-1	IVP97-079-9	IVP08-206-32	AGV09-007-13									
	PotSNP834	C:C	T:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	PotSNP392	T:T	T:A	T:A	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	PotSNp038	C:C	C:T	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	PotSNP947	T:T	T:G	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	PotSNP985	G:G	A:A	A:G	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
	PotSNP796	C:C	C:A	C:A	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	PotSNP569	G:G	A:A	G:A	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	PotSNP928	T:T	A:A	A:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	PotSNP184	G:G	G:A	G:A	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	PotSNP1105	A:A	C:C	A:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	PotSNP205	A:A	G:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
	PotSNP626	G:G	A:A	G:A	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	PotSNP229	C:C	C:T	C:T	C:T	C:T	C:T	C:C	C:T	T:T	T:T	C:T	C:T	C:T
	PotSNP138	T:T	C:T	G:A	A:A	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	PotSNP1026	G:G	G:C	A:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	PotSNP700	G:G	G:A	?	?	G:G	G:G	G:G	G:G	G:G	?	G:G	G:G	G:G
	PotSNP102	A:A	G:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
	PotSNP458	G:G	G:A	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	PotSNP021	C:C	T:T	?	?	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	PotSNP152	C:C	C:T	C:T	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	PotSNP120	A:A	G:A	G:A	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	PotSNP607	C:C	T:C	T:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	PotSNP580	C:C	T:T	T:C	T:C	T:C	C:C	T:C	T:T	T:C	T:T	T:C	T:C	C:C
	PotSNP099	C:C	C:T	C:T	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	PotSNP182	A:A	G:A	G:A	G:A	G:A	A:A	?	G:A	A:A	A:A	G:A	G:G	A:A
	PotSNP052	C:C	C:T	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C

TO FIG. 5C-3



123-26	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	T:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	C:C	C:C	C:C	G:A	C:C
123-25	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	C:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	T:C	C:C	?	C:C
123-24	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	C:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	C:C	C:C	G:A	C:C
123-23	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	T:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	C:C	C:C	G:G	C:C
123-22	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	C:C	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	C:C	C:C	A:A	C:C
123-21	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	C:C	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	T:C	C:C	G:A	C:C
123-20	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	T:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	C:C	C:C	A:A	C:C
123-19	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	C:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	C:C	C:C	A:A	C:C
123-18	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	C:C	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	T:C	C:C	G:A	C:C
123-17	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	T:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	T:C	C:C	G:A	C:C
123-16	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	C:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	C:C	C:C	G:A	C:C
123-15	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	C:C	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	T:C	C:C	A:A	C:C
123-14	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	T:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	T:C	C:C	G:A	C:C
123-13	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	C:C	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	C:C	C:C	G:A	C:C
123-12	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	C:C	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	T:C	C:C	G:A	C:C
123-11	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	C:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	T:C	C:C	G:A	C:C
123-10	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	C:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	T:C	C:C	G:A	C:C

FIG. 5C-2

FROM FIG. 5C-1

FIG. 5C-3

123-35	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	T:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	T:C	C:C	G:A	C:C
123-34	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	C:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	C:C	C:C	G:A	C:C
123-33	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	T:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	T:T	C:C	G:A	C:C
123-32	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	C:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	C:C	C:C	G:G	C:C
123-31	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	C:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	T:C	C:C	G:A	C:C
123-30	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	C:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	C:C	C:C	G:A	C:C
123-29	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	T:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	T:C	C:C	G:A	C:C
123-28	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	T:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	T:C	C:C	G:A	C:C
123-27	C:C	T:T	C:C	T:T	A:A	C:C	G:G	T:T	G:G	C:C	A:A	G:G	C:T	T:T	G:G	G:G	A:A	G:G	C:C	C:C	G:G	C:C	T:T	C:C	G:A	C:C

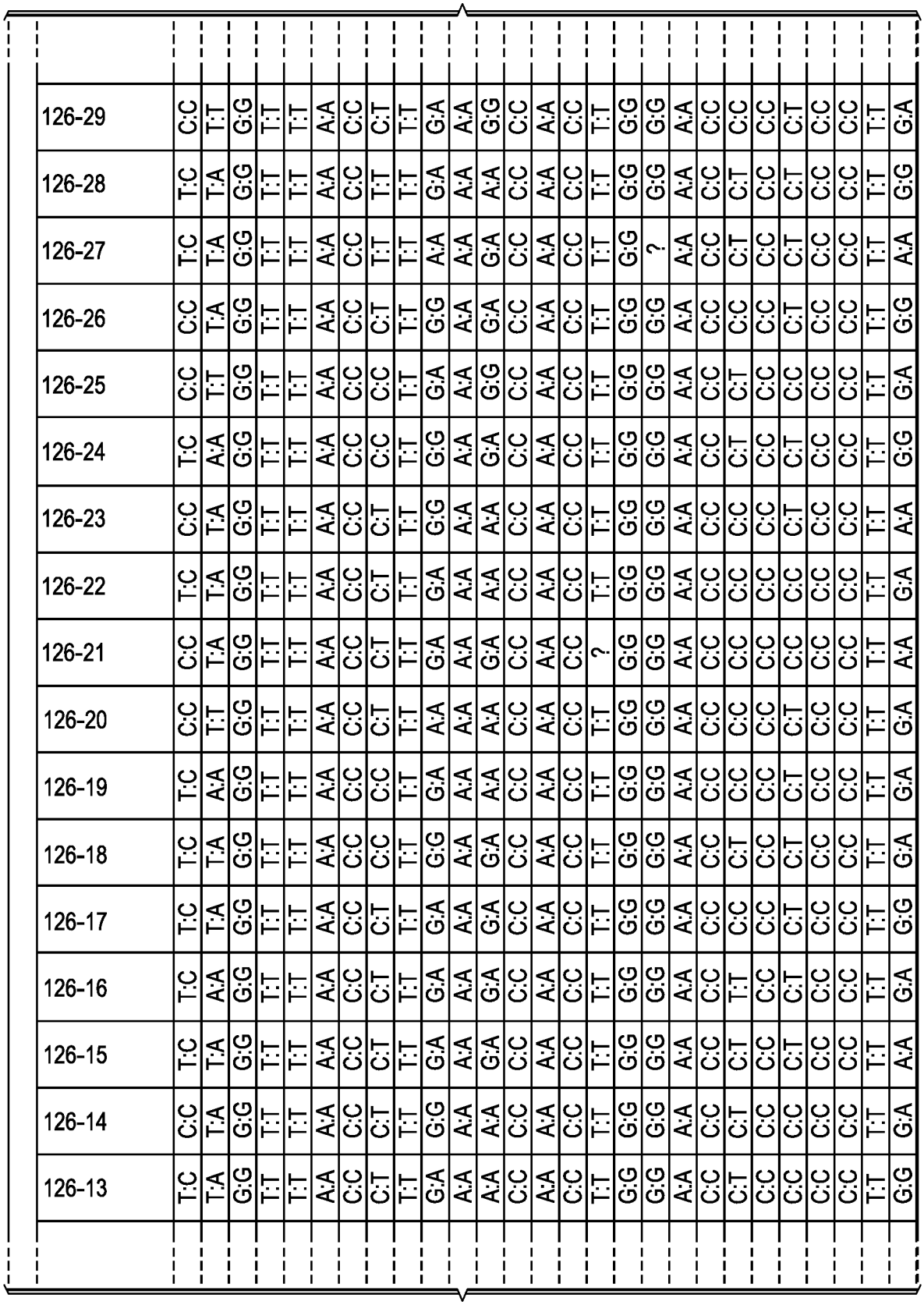
FROM FIG. 5C-2

TO FIG. 5D-2

					F3 populatie 126										
	SNP	H	D2	F1	F2	126-1	126-2	126-3	126-4	126-5	126-6	126-7	126-8	126-9	126-10	126-11	126-12
		07-1004-1	IVPAA-096-18	IVP08-207-20	AGV09-008-16												
	PotSNP834	C:C	T:T	T:C	T:C	T:C	T:C	C:C	T:T	T:C	T:C	T:C	T:C	T:C	T:C	T:T	T:C
	PotSNP392	T:T	T:A		T:A	A:A	A:A	?	T:A	T:A	A:A	T:A	T:A	T:A	T:A	A:A	A:A
	PotSNP165	G:G	G:A	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	PotSNp038	C:C	C:T	C:T	C:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	PotSNP947	T:T	T:G	T:G	T:G	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	PotSNP985	G:G	A:A	A:G	A:G	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
	PotSNP796	C:C	C:A	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	PotSNP960	C:C	C:T	C:T	C:C	C:T	C:C	C:T	C:T	?	C:T	C:T	C:C	C:T	C:T	C:T	C:C
	PotSNP928	T:T	A:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	?	T:T
	PotSNP184	G:G	A:A	G:A	G:G	G:A	G:A	G:A	G:G	G:A	G:A	G:G	G:A	G:G	G:A	G:G	G:A
	PotSNP1105	A:A	A:C	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
	PotSNP753	A:A	G:A	G:A	G:G	G:A	G:A	G:A	G:A	A:A	A:A	G:A	A:A	?	?	G:A	G:A
	PotSNP081	C:C	C:T	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	PotSNP124	A:A	G:A	G:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
	PotSNP229	C:C	C:T		C:T	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	PotSNP138	T:T	C:T	C:T	?	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	PotSNP700	G:G	A:A	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	PotSNP116	G:G	G:A	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	PotSNP102	A:A	G:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
	PotSNP162	T:T	C:T	?	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	PotSNP021	C:C	T:T	C:T	T:T	T:T	C:T	C:C	C:T	C:C	T:T	C:T	C:C	C:C	C:T	T:T	C:T
	PotSNP152	C:C	C:T	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	PotSNP100	C:C	C:T	C:T	C:T	C:T	C:C	C:T	C:C	C:T	C:T	C:T	C:C	C:T	C:T	C:C	C:T
	PotSNP580	C:C	T:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	PotSNP099	C:C	T:T		C:T	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	PotSNP996	T:T	C:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	PotSNP213	G:G	?	G:A	G:A	A:A	G:G	G:A	G:A	G:A	G:G	G:A	G:A	?	G:A	A:A	A:A

FIG. 5D-1

TO FIG. 5D-3

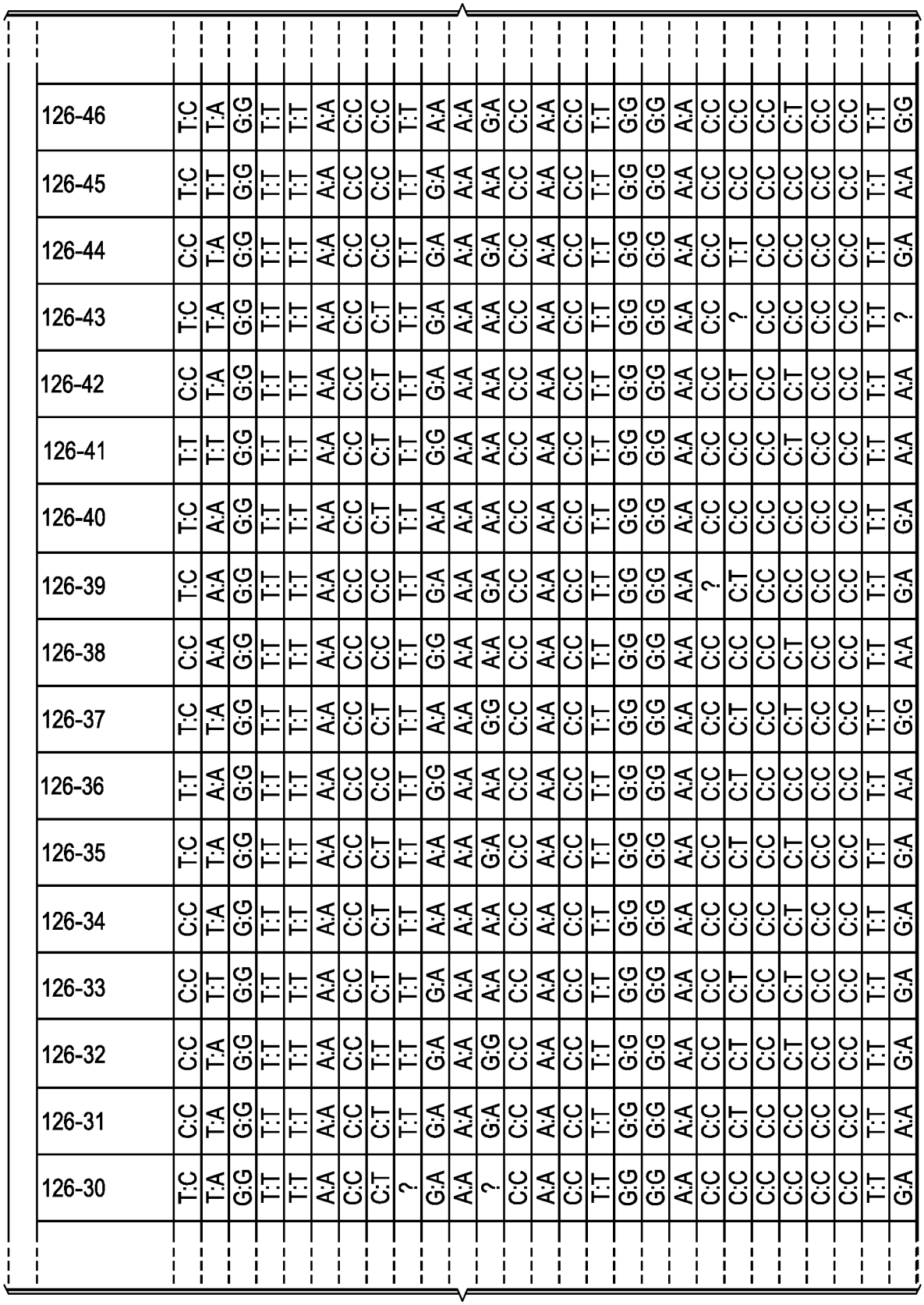


126-29	C:C	T:T	G:G	T:T	T:T	A:A	C:C	C:T	T:T	G:A	A:A	G:G	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:C	C:C	C:T	C:C	C:C	T:T	G:A
126-28	T:C	T:A	G:G	T:T	T:T	A:A	C:C	T:T	T:T	G:A	A:A	A:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:T	C:C	C:T	C:C	C:C	T:T	G:G
126-27	T:C	T:A	G:G	T:T	T:T	A:A	C:C	T:T	T:T	A:A	A:A	G:A	C:C	A:A	C:C	T:T	G:G	?	A:A	C:C	C:T	C:C	C:T	C:C	C:C	T:T	A:A
126-26	C:C	T:A	G:G	T:T	T:T	A:A	C:C	C:T	T:T	G:G	A:A	G:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:C	C:C	C:T	C:C	C:C	T:T	G:G
126-25	C:C	T:T	G:G	T:T	T:T	A:A	C:C	C:C	T:T	G:A	A:A	G:G	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:T	C:C	C:C	C:C	C:C	T:T	G:A
126-24	T:C	A:A	G:G	T:T	T:T	A:A	C:C	C:C	T:T	G:G	A:A	G:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:T	C:C	C:C	C:C	C:C	T:T	G:G
126-23	C:C	T:A	G:G	T:T	T:T	A:A	C:C	C:T	T:T	G:G	A:A	A:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:C	C:C	C:T	C:C	C:C	T:T	A:A
126-22	T:C	T:A	G:G	T:T	T:T	A:A	C:C	C:T	T:T	G:A	A:A	A:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:C	C:C	C:C	C:C	C:C	T:T	G:A
126-21	C:C	T:A	G:G	T:T	T:T	A:A	C:C	C:T	T:T	G:A	A:A	G:A	C:C	A:A	C:C	?	G:G	G:G	A:A	C:C	C:C	C:C	C:C	C:C	C:C	T:T	A:A
126-20	C:C	T:T	G:G	T:T	T:T	A:A	C:C	C:T	T:T	A:A	A:A	A:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:C	C:C	C:T	C:C	C:C	T:T	G:A
126-19	T:C	A:A	G:G	T:T	T:T	A:A	C:C	C:C	T:T	G:A	A:A	A:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:C	C:C	C:T	C:C	C:C	T:T	G:A
126-18	T:C	T:A	G:G	T:T	T:T	A:A	C:C	C:C	T:T	G:G	A:A	G:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:T	C:C	C:T	C:C	C:C	T:T	G:A
126-17	T:C	T:A	G:G	T:T	T:T	A:A	C:C	C:T	T:T	G:A	A:A	G:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:C	C:C	C:T	C:C	C:C	T:T	G:G
126-16	T:C	A:A	G:G	T:T	T:T	A:A	C:C	C:T	T:T	G:A	A:A	G:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	T:T	C:C	C:T	C:C	C:C	T:T	G:A
126-15	T:C	T:A	G:G	T:T	T:T	A:A	C:C	C:T	T:T	G:A	A:A	G:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:T	C:C	C:T	C:C	C:C	T:T	A:A
126-14	C:C	T:A	G:G	T:T	T:T	A:A	C:C	C:T	T:T	G:G	A:A	A:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:T	C:C	C:T	C:C	C:C	T:T	G:A
126-13	T:C	T:A	G:G	T:T	T:T	A:A	C:C	C:T	T:T	G:A	A:A	A:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:T	C:C	C:C	C:C	C:C	T:T	G:G

FROM FIG. 5D-1

FIG. 5D-2

TO FIG. 5D-4



	126-46	126-45	126-44	126-43	126-42	126-41	126-40	126-39	126-38	126-37	126-36	126-35	126-34	126-33	126-32	126-31	126-30
	T:C	T:C	C:C	T:C	C:C	T:T	T:C	T:C	C:C	T:C	T:T	T:C	C:C	C:C	C:C	C:C	T:C
	T:A	T:T	T:A	T:A	T:A	T:T	A:A	A:A	A:A	T:A	A:A	T:A	T:A	T:T	T:A	T:A	T:A
	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	C:C	C:C	C:C	C:T	C:T	C:T	C:T	C:C	C:C	C:T	C:C	C:T	C:C	C:C	C:C	C:T	C:C
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	?
	G:A	G:A	G:A	G:A	G:A	G:G	A:A	G:A	G:G	A:A	G:G	A:A	A:A	G:A	G:A	G:A	G:A
	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	?
	A:A	A:A	G:A	A:A	A:A	A:A	A:A	G:A	A:A	G:G	A:A	G:A	A:A	A:A	G:G	A:A	G:A
	G:A	A:A	G:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	C:T	C:C	C:C	C:C	C:T	C:C	C:A	C:A	C:A	G:G	C:C	G:A	G:A	G:A	G:A	A:A	G:A
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	G:A	A:A	G:A	?	A:A	A:A	G:A	G:A	A:A	G:G	A:A	G:A	G:A	G:A	G:A	A:A	G:A
	G:G	G:G	G:A	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	C:T	C:C	C:C	C:C	C:T	C:C	C:C	C:C	C:C	C:G	C:C	C:A	C:A	C:A	C:A	C:A	G:A
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	G:G	A:A	G:A	?	A:A	A:A	G:A	G:A	A:A	G:G	A:A	G:A	G:A	G:A	G:A	A:A	G:A

FIG. 5D-3

FROM FIG. 5D-2

FIG. 5D-4

126-56	C:C	T:T	G:G	T:T	T:T	A:A	C:C	C:T	T:T	G:A	A:A	A:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:C	C:C	C:T	C:C	C:C	T:T	G:A
126-55	T:C	T:A	G:G	T:T	T:T	A:A	C:C	C:T	T:T	G:G	A:A	A:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:T	C:C	C:T	C:C	C:C	T:T	G:G
126-54	T:C	T:A	G:G	T:T	T:T	A:A	C:C	T:T	T:T	A:A	A:A	A:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:T	C:C	C:C	C:C	C:C	T:T	A:A
126-53	T:C	T:A	G:G	T:T	T:T	A:A	C:C	T:T	T:T	A:A	A:A	A:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:T	C:C	C:C	C:C	C:C	T:T	A:A
126-52	T:C	A:A	G:G	T:T	T:T	A:A	C:C	C:C	T:T	A:A	A:A	A:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	T:T	C:C	C:T	C:C	C:C	T:T	G:A
126-51	C:C	T:A	G:G	T:T	T:T	A:A	C:C	C:C	T:T	A:A	A:A	G:G	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:T	C:C	C:T	C:C	C:C	T:T	G:A
126-50	C:C	T:T	G:G	T:T	T:T	A:A	C:C	C:C	T:T	G:G	A:A	A:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:C	C:C	C:T	C:C	C:C	T:T	G:A
126-49	T:C	T:T	G:G	T:T	T:T	A:A	C:C	C:T	T:T	A:A	A:A	A:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:T	C:C	C:T	C:C	C:C	T:T	G:G
126-48	T:C	A:A	G:G	T:T	T:T	A:A	C:C	T:T	T:T	G:A	A:A	A:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:C	C:C	C:T	C:C	C:C	T:T	G:A
126-47	C:C	T:A	G:G	T:T	T:T	A:A	C:C	T:T	T:T	G:A	A:A	A:A	C:C	A:A	C:C	T:T	G:G	G:G	A:A	C:C	C:T	C:C	C:T	C:C	C:C	T:T	G:A

FROM FIG. 5D-3

TO FIG. 5E-2

					F3 populatie 126B										
SNP	H	D2	F1	F2		126B-1	126B-2	126B-3	126B-4	126B-5	126B-6	126B-7	126B-8	126B-9	126B-10	126B-11	126B-12
PotSNP834	C:C	T:T	T:C	T:C		T:C	T:C	T:C	T:C	T:C	C:C	T:C	T:T	C:C	C:C	C:C	T:C
PotSNP165	G:G	G:A	G:G	G:G		G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
PotSNp038	C:C	C:T	C:T	C:T		C:T	C:T	C:C	C:T	C:T	C:C	C:T	C:T	C:C	T:T	C:T	C:T
PotSNP947	T:T	T:G	T:G	T:G		T:G	T:T	G:G	G:G	G:G	T:T	T:G	T:G	G:G	T:G	G:G	G:G
PotSNP985	G:G	A:A	A:G	A:G		A:G	A:G	A:A	A:A	A:G	G:G	A:G	A:G	A:G	A:A	G:G	A:A
PotSNP796	C:C	C:A	C:C	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
PotSNP960	C:C	C:T	C:T	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
PotSNP928	T:T	A:T	T:T	T:T		T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
PotSNP184	G:G	A:A	G:A	G:G		G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
PotSNP1105	A:A	A:C	A:A	A:A		A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
PotSNP753	A:A	G:A	G:A	G:G		G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
PotSNP081	C:C	C:T	C:C	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
PotSNP124	A:A	G:A	G:A	A:A		A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	?	A:A	A:A	A:A
PotSNP700	G:G	A:A	G:G	G:G		?	G:G	G:G	G:G	G:G	?	G:G	G:G	G:G	G:G	G:G	G:G
PotSNP116	G:G	G:A	G:G	G:G		G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
PotSNP102	A:A	G:A	A:A	A:A		A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
PotSNP162	T:T	C:T	?	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
PotSNP021	C:C	T:T	C:T	T:T		T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
PotSNP152	C:C	C:T	C:C	C:C		C:C	C:C	C:C	C:C	C:C	?	C:C	C:C	C:C	C:C	C:C	C:C
PotSNP100	C:C	C:T	C:T	C:T		C:T	C:C	C:T	C:T	C:T	C:T	C:C	C:T	C:T	C:C	C:T	C:T
PotSNP580	C:C	T:C	C:C	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
PotSNP996	T:T	C:T	T:T	T:T		T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
PotSNP213	G:G	?	G:A	G:A		G:A	G:G	G:G	G:G	G:A	G:A	G:G	G:A	G:G	A:A	G:G	G:A
PotSNP052	C:C	C:T	C:C	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C

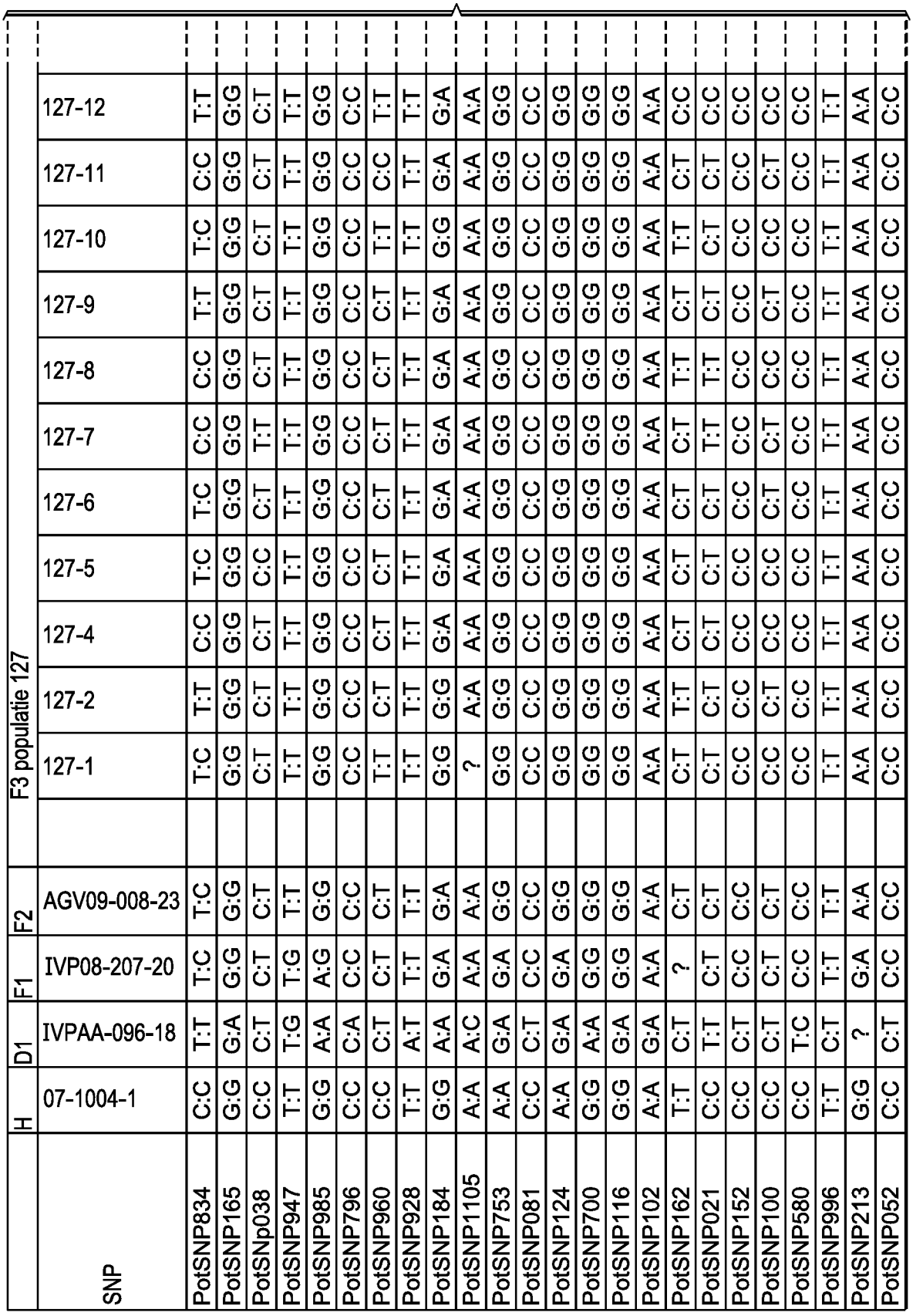
FIG. 5E-1

FIG. 5E-2

126-31	C:C	G:G	T:T	T:G	A:A	C:C	C:C	T:T	G:G	A:A	G:G	C:C	A:A	G:G	G:G	A:A	C:C	T:T	C:C	C:C	T:T	C:C	C:C	T:T	A:A	C:C
126-30	T:C	G:G	C:T	T:T	A:A	C:C	C:C	T:T	G:G	A:A	G:G	C:C	A:A	G:G	G:G	A:A	C:C	T:T	C:C	C:C	C:T	C:C	C:C	T:T	A:A	C:C
126-29	T:C	G:G	C:T	T:G	G:G	C:C	C:C	T:T	G:G	A:A	G:G	C:C	A:A	G:G	G:G	A:A	C:C	T:T	C:C	C:C	C:C	C:C	C:C	T:T	A:A	C:C
126-28	T:C	G:G	T:T	T:G	A:G	C:C	C:C	T:T	G:G	A:A	G:G	C:C	A:A	G:G	G:G	A:A	C:C	T:T	C:C	C:C	C:T	C:C	C:C	T:T	G:G	C:C
126-27	T:T	G:G	C:C	T:G	G:G	C:C	C:C	T:T	G:G	A:A	G:G	C:C	A:A	G:G	G:G	A:A	C:C	T:T	C:C	C:C	C:T	C:C	C:C	T:T	G:G	C:C
126-26	C:C	G:G	T:T	G:G	A:G	C:C	C:C	T:T	G:G	A:A	G:G	C:C	A:A	G:G	G:G	A:A	C:C	T:T	C:C	C:C	C:C	C:C	C:C	T:T	G:G	C:C
126-25	C:C	G:G	C:T	G:G	?	C:C	?	T:T	G:G	A:A	G:G	C:C	?	G:G	G:G	A:A	C:C	T:T	C:C	C:C	C:T	C:C	C:C	T:T	G:A	C:C
126-24	T:C	G:G	C:T	T:G	A:G	C:C	C:C	T:T	G:G	A:A	G:G	C:C	A:A	G:G	G:G	A:A	C:C	T:T	C:C	C:C	C:T	C:C	C:C	T:T	G:A	C:C
126-23	C:C	G:G	C:T	T:T	A:G	C:C	C:C	T:T	G:G	A:A	G:G	C:C	A:A	G:G	G:G	A:A	C:C	T:T	C:C	C:C	C:T	C:C	C:C	T:T	A:A	C:C
126-22	T:C	G:G	T:T	T:G	A:A	C:C	C:C	T:T	G:G	A:A	G:G	C:C	A:A	G:G	G:G	A:A	C:C	T:T	C:C	C:C	T:T	C:C	C:C	T:T	G:A	C:C
126-21	T:C	G:G	C:C	G:G	A:G	C:C	C:C	T:T	G:G	?	G:G	C:C	A:A	G:G	G:G	A:A	C:C	T:T	C:C	C:C	C:T	C:C	C:C	T:T	A:A	C:C
126-20	T:C	G:G	T:T	G:G	A:G	C:C	C:C	T:T	G:G	A:A	G:G	C:C	A:A	G:G	G:G	A:A	C:C	T:T	C:C	C:C	C:C	C:C	C:C	T:T	G:A	C:C
126-19	C:C	G:G	C:T	T:G	A:G	C:C	C:C	T:T	G:G	A:A	G:G	C:C	A:A	G:G	G:G	A:A	C:C	T:T	C:C	C:C	C:C	C:C	C:C	T:T	A:A	C:C
126-18	T:C	G:G	C:T	G:G	A:G	C:C	C:C	T:T	G:G	A:A	G:G	C:C	A:A	G:G	G:G	A:A	C:C	T:T	C:C	C:C	C:T	C:C	C:C	T:T	G:G	C:C
126-17	T:T	G:G	T:T	G:G	G:G	C:C	C:C	T:T	G:G	A:A	G:G	C:C	A:A	G:G	G:G	A:A	C:C	T:T	C:C	C:C	C:C	C:C	C:C	T:T	A:A	C:C
126-16	C:C	G:G	C:C	T:T	A:G	C:C	C:C	T:T	G:G	A:A	G:G	C:C	A:A	G:G	G:G	A:A	C:C	T:T	C:C	C:C	C:C	C:C	C:C	T:T	A:A	C:C
126-15	T:C	G:G	C:T	T:T	G:G	C:C	C:C	T:T	G:G	A:A	G:G	C:C	A:A	G:G	G:G	A:A	C:C	T:T	C:C	C:C	C:C	C:C	C:C	T:T	A:A	C:C
126-14	T:T	G:G	C:T	T:G	A:G	C:C	C:C	T:T	G:G	A:A	G:G	C:C	A:A	G:G	G:G	A:A	C:C	T:T	C:C	C:C	C:C	C:C	C:C	T:T	G:A	C:C
126-13	C:C	G:G	T:T	T:T	A:G	C:C	C:C	T:T	G:G	A:A	G:G	C:C	A:A	G:G	G:G	A:A	C:C	T:T	C:C	C:T	C:C	C:C	T:T	G:G	C:C	

FROM FIG. 5E-1

TO FIG. 5F-2



		H	D1	F1	F2	F3 populatie 127											
	SNP	07-1004-1	IVPAA-096-18	IVP08-207-20	AGV09-008-23		127-1	127-2	127-4	127-5	127-6	127-7	127-8	127-9	127-10	127-11	127-12
	PotSNP834	C:C	T:T	T:C	T:C		T:C	T:T	C:C	T:C	T:C	C:C	C:C	T:T	T:C	C:C	T:T
	PotSNP165	G:G	G:A	G:G	G:G		G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	PotSNp038	C:C	C:T	C:T	C:T		C:T	C:T	C:T	C:C	C:T	T:T	C:T	C:T	C:T	C:T	C:T
	PotSNP947	T:T	T:G	T:G	T:T		T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	PotSNP985	G:G	A:A	A:G	G:G		G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	PotSNP796	C:C	C:A	C:C	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	PotSNP960	C:C	C:T	C:T	C:T		T:T	C:T	C:T	C:T	C:T	C:T	C:T	C:T	T:T	C:C	T:T
	PotSNP928	T:T	A:T	T:T	T:T		T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	PotSNP184	G:G	A:A	G:A	G:A		G:G	G:G	G:A	G:A	G:A	G:A	G:A	G:A	G:G	G:A	G:A
	PotSNP1105	A:A	A:C	A:A	A:A		?	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
	PotSNP753	A:A	G:A	G:A	G:G		G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	PotSNP081	C:C	C:T	C:C	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	PotSNP124	A:A	G:A	G:A	G:G		G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	PotSNP700	G:G	A:A	G:G	G:G		G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	PotSNP116	G:G	G:A	G:G	G:G		G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G	G:G
	PotSNP102	A:A	G:A	A:A	A:A		A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
	PotSNP162	T:T	C:T	?	C:T		C:T	T:T	C:T	C:T	C:T	C:T	T:T	C:T	T:T	C:T	C:C
	PotSNP021	C:C	T:T	C:T	C:T		C:T	C:T	C:T	C:T	C:T	C:T	T:T	C:T	C:T	C:T	C:C
	PotSNP152	C:C	C:T	C:C	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	PotSNP100	C:C	C:T	C:T	C:T		C:C	C:T	C:C	C:C	C:T	C:T	C:C	C:T	C:C	C:T	C:C
	PotSNP580	C:C	T:C	C:C	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
	PotSNP996	T:T	C:T	T:T	T:T		T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T
	PotSNP213	G:G	?	G:A	A:A		A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A	A:A
	PotSNP052	C:C	C:T	C:C	C:C		C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C

FIG. 5F-1

REPLACEMENT SHEET

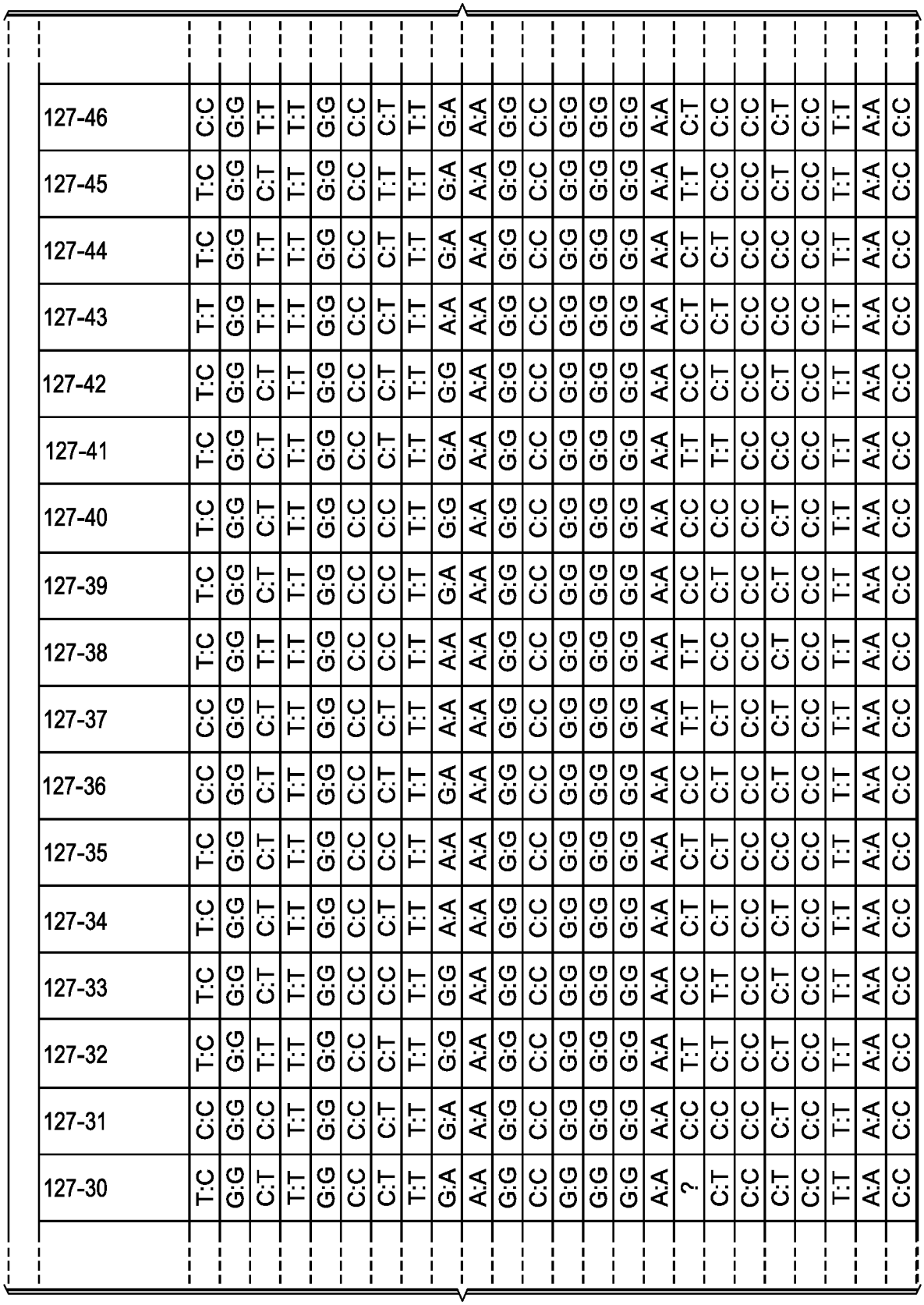
FIG. 5F-2

TO FIG. 5F-3

127-29	T:C	G:G	C:T	T:T	G:G	C:C	C:T	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	C:T	C:C	C:T	C:C	T:T	A:A	C:C
127-28	C:C	G:G	C:T	T:T	G:G	C:C	C:C	T:T	A:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	C:T	C:C	C:T	C:C	T:T	A:A	C:C
127-27	T:C	G:G	C:T	T:T	G:G	C:C	T:T	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	C:C	C:C	C:C	C:C	T:T	A:A	C:C
127-26	T:C	G:G	T:T	T:T	G:G	C:C	C:C	T:T	A:A	A:A	G:G	C:C	G:G	G:G	?	A:A	C:T	C:C	C:C	C:C	C:C	T:T	A:A	C:C
127-25	T:C	G:G	C:T	T:T	G:G	C:C	C:C	T:T	G:G	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:C	T:T	C:C	C:T	C:C	T:T	A:A	C:C
127-24	C:C	G:G	C:C	T:T	G:G	C:C	T:T	T:T	G:G	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	C:C	C:C	C:T	C:C	T:T	A:A	C:C
127-23	T:T	G:G	T:T	T:T	G:G	C:C	T:T	T:T	A:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	T:T	C:C	C:C	C:C	T:T	A:A	C:C
127-22	T:C	G:G	T:T	T:T	G:G	C:C	C:C	T:T	G:G	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	C:C	C:C	C:C	C:C	T:T	A:A	C:C
127-21	T:C	G:G	T:T	T:T	G:G	C:C	C:T	T:T	G:G	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	T:T	C:C	C:C	C:C	T:T	A:A	C:C
127-20	T:T	G:G	C:T	T:T	G:G	C:C	T:T	T:T	A:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	C:C	C:C	C:T	C:C	T:T	A:A	C:C
127-19	C:C	G:G	C:T	T:T	G:G	C:C	C:C	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	?	C:C	C:T	C:C	C:T	C:C	T:T	A:A	C:C
127-18	C:C	G:G	T:T	T:T	G:G	C:C	T:T	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	C:T	C:C	C:T	C:C	T:T	A:A	C:C
127-17	C:C	G:G	C:T	T:T	G:G	C:C	C:T	T:T	G:G	A:A	G:G	C:C	G:G	G:G	G:G	A:A	T:T	T:T	C:C	C:T	C:C	T:T	A:A	C:C
127-16	C:C	G:G	C:T	T:T	G:G	C:C	C:T	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	C:T	C:C	C:T	C:C	T:T	A:A	C:C
127-15	T:T	G:G	T:T	T:T	G:G	C:C	C:T	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:C	T:T	C:C	C:C	C:C	T:T	A:A	C:C
127-14	T:C	G:G	C:C	T:T	G:G	C:C	C:T	T:T	G:G	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	T:T	C:C	C:C	C:C	T:T	A:A	C:C
127-13	T:T	G:G	T:T	T:T	G:G	C:C	T:T	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	T:T	C:C	C:T	C:C	T:T	A:A	C:C

FROM FIG. 5F-1

TO FIG. 5F-4



127-46	C:C	G:G	T:T	T:T	G:G	C:C	C:T	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	C:C	C:C	C:T	C:C	T:T	A:A	C:C
127-45	T:C	G:G	C:T	T:T	G:G	C:C	T:T	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	T:T	C:C	C:C	C:T	C:C	T:T	A:A	C:C
127-44	T:C	G:G	T:T	T:T	G:G	C:C	C:T	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	C:T	C:C	C:C	C:C	T:T	A:A	C:C
127-43	T:T	G:G	T:T	T:T	G:G	C:C	C:T	T:T	A:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	C:T	C:C	C:C	C:C	T:T	A:A	C:C
127-42	T:C	G:G	C:T	T:T	G:G	C:C	C:T	T:T	G:G	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:C	C:T	C:C	C:T	C:C	T:T	A:A	C:C
127-41	T:C	G:G	C:T	T:T	G:G	C:C	C:T	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	T:T	T:T	C:C	C:C	C:C	T:T	A:A	C:C
127-40	T:C	G:G	C:T	T:T	G:G	C:C	C:C	T:T	G:G	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:C	C:C	C:C	C:T	C:C	T:T	A:A	C:C
127-39	T:C	G:G	C:T	T:T	G:G	C:C	C:C	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:C	C:T	C:C	C:T	C:C	T:T	A:A	C:C
127-38	T:C	G:G	T:T	T:T	G:G	C:C	C:C	T:T	A:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	T:T	C:C	C:C	C:T	C:C	T:T	A:A	C:C
127-37	C:C	G:G	C:T	T:T	G:G	C:C	C:T	T:T	A:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	T:T	C:T	C:C	C:T	C:C	T:T	A:A	C:C
127-36	C:C	G:G	C:T	T:T	G:G	C:C	C:T	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:C	C:T	C:C	C:T	C:C	T:T	A:A	C:C
127-35	T:C	G:G	C:T	T:T	G:G	C:C	C:C	T:T	A:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	C:T	C:C	C:T	C:C	T:T	A:A	C:C
127-34	T:C	G:G	C:T	T:T	G:G	C:C	C:T	T:T	A:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	C:T	C:C	C:T	C:C	T:T	A:A	C:C
127-33	T:C	G:G	C:T	T:T	G:G	C:C	C:C	T:T	G:G	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:C	T:T	C:C	C:T	C:C	T:T	A:A	C:C
127-32	T:C	G:G	T:T	T:T	G:G	C:C	C:T	T:T	G:G	A:A	G:G	C:C	G:G	G:G	G:G	A:A	T:T	C:T	C:C	C:T	C:C	T:T	A:A	C:C
127-31	C:C	G:G	C:C	T:T	G:G	C:C	C:T	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:C	C:C	C:C	C:T	C:C	T:T	A:A	C:C
127-30	T:C	G:G	C:T	T:T	G:G	C:C	C:T	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	?	C:T	C:C	C:T	C:C	T:T	A:A	C:C

FROM FIG. 5F-2

FIG. 5F-3

FIG. 5F-4

127-56	C:C	G:G	C:T	T:T	G:G	C:C	C:T	T:T	A:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	C:T	C:C	C:T	C:C	T:T	A:A	C:C
127-55	C:C	G:G	C:C	T:T	G:G	C:C	?	T:T	A:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	C:T	C:C	?	C:C	T:T	A:A	C:C
127-54	T:C	G:G	T:T	T:T	G:G	C:C	C:T	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	T:T	T:T	C:C	C:C	C:C	T:T	A:A	C:C
127-53	T:C	G:G	C:C	T:T	G:G	C:C	C:T	T:T	G:G	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:C	T:T	C:C	C:C	C:C	T:T	A:A	C:C
127-52	T:C	G:G	T:T	T:T	G:G	C:C	C:C	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	T:T	C:C	C:C	C:C	T:T	A:A	C:C
127-51	T:C	G:G	C:T	T:T	G:G	C:C	T:T	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	T:T	C:C	C:C	C:C	T:T	A:A	C:C
127-50	C:C	G:G	C:C	T:T	G:G	C:C	C:T	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	C:T	C:C	C:T	C:C	T:T	A:A	C:C
127-49	T:C	G:G	T:T	T:T	G:G	C:C	T:T	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:C	T:T	C:C	C:C	C:C	T:T	A:A	C:C
127-48	T:C	G:G	C:C	T:T	G:G	C:C	T:T	T:T	G:G	A:A	G:G	C:C	G:G	G:G	G:G	A:A	C:T	C:T	C:C	C:T	C:C	T:T	A:A	C:C
127-47	T:C	G:G	C:T	T:T	G:G	C:C	C:T	T:T	G:A	A:A	G:G	C:C	G:G	G:G	G:G	A:A	T:T	C:T	C:C	C:T	C:C	T:T	A:A	C:C

FROM FIG. 5F-3

MARKED
SUBSTITUTE SPECIFICATION

Title: Hybrid Seed Potato Breeding

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of United States Application No. 13/504,156, filed July 10, 2012 (pending), which application is a 371 National Stage application of PCT/NL10/50716 which application claims the benefit of priority to United States Provisional Application No. 61/254,980, filed October 26, 2009, and to European Patent Application No. 09013474.3, filed on October 26, 2009, each of which are herein incorporated by reference in their entireties.

FIELD OF THE INVENTION

The invention relates to the field of agriculture, in particular to new plants and processes for obtaining them. The invention also relates to methods for improving the genetic constitution of crop plants, and to plants thus obtained. The invention further relates to a method for producing hybrid seeds and to hybrid seeds thus produced and to methods of producing crops using the hybrid seeds of the invention.

BACKGROUND OF THE INVENTION

The potato (*Solanum tuberosum* L.) is an integral part of the global food system. It is the world's number one non-grain food commodity, with production reaching a record 325 million tonnes in 2007.

Unlike other major field crops, potatoes are reproduced vegetatively from other potatoes. Therefore, a part of each year's crop - ranging from 5 to 15 percent, depending on the quality of the harvested tubers - is set aside for re-use in the next planting season. Most farmers in developing countries select and store their own seed tubers. In developed countries, farmers are more likely to purchase disease-free "certified seed" from dedicated suppliers.

Seed potatoes are more difficult to produce and supply than grain or pulse seed. A seed:harvest ratio of 1:20 for potatoes is considered good, compared to 1:400 for maize or 1:10.000 for tomato. One hectare may therefore require two tonnes of seed material to maximize the yield of harvestable products, compared to 18 kg for maize. In order to break dormancy, seed potatoes should be stored for several weeks before they can be planted. The right conditions during storage such as amount of light, temperature and humidity are crucial to ensure good "seed" quality.

In addition to the poor seed:harvest ratio, seed potatoes attract and transport pests and diseases. These include (amongst others) late blight, Andean potato weevils, nematodes, tuber moths and viruses. The latter are transmitted in the field by aphids and then carried from generation to generation in the seed. Such a virus infection can decrease yields by up to 20 percent.

Seed potatoes have high transportation costs because of the great distances between the major seed production areas and the major consumer production areas and the relatively high weight of individual seed potato tubers.

To feed the growing world population now and in future, the potato industry has to keep growing to meet the needs of the consuming public. Substantial research and development efforts are devoted to the modernization of planting and harvesting of fields and processing of potatoes, and to the development of economically advantageous potato varieties. Through crossbreeding of potatoes, researchers hope to obtain potatoes with the desirable characteristics of good processing, both for fresh consumption as well as for industrial purposes, high soluble solids content, high yield, resistance to diseases and pests and adaptability to various growing areas and conditions.

The research leading to potato varieties which combine the advantageous characteristics referred to above is largely empirical. This research requires large investments of time, manpower, and money. The development of a potato cultivar can often take up to eight years or more followed by at least five years of propagation to get sufficient quantities for commercial usage. Breeding begins with careful selection of superior parents to incorporate the most important characteristics into the progeny. Since all desired traits usually do not appear in one progeny, breeding is a continuous process of selecting the best recombinants, combining favourable traits of the ancestors.

The arduous task of producing a new potato variety is best understood when understanding the genetics of potato. The commercial potato has a tetraploid genome. Diploid tubers are generally too small for important commercial applications. In addition, the tetraploid genome is extremely heterozygous, often harbouring multiple alleles per locus. It is believed that self-incompatibility, which is primarily experienced at diploid level, and inbreeding depression are responsible for the maintenance of the high genetic variability found in potato and that overdominance of heterozygous alleles (heterosis) results in vigorous plants. In a typical potato offspring obtained from a cross between two unrelated parental lines deleterious alleles may therefore contribute to either a reduced fitness in case of homozygosity or an increased vigour in case of heterozygosity. It is clear that a breeder needs large populations to maximise the chance of finding individuals that

carry a relatively high number of heterozygotic loci and a low number of homozygotic loci, while also exhibiting beneficial combinations of agronomically desirable traits.

Present potato breeding techniques rely on the controlled crossing of parental clones which themselves are the result of a comprehensive pre-breeding development during which amongst others special techniques such as chromosome doubling, embryo rescue, and somatic fusion are applied in order to introduce the beneficial characteristics of for instance wild and primitive *Solanum* species into these clones. The parental material that is found suitable for further breeding after a phenotypic selection procedure is then mutually crossed and the resulting non-uniform hybrid seeds are sown in large numbers in greenhouses. From tens of thousands of individual F1 seedlings tubers are harvested and retained for the next year's planting. The next year a single "seed" tuber from each resulting seedling is planted in the field. Extreme caution must be taken to avoid the introduction of viruses and diseases since the material is only clonally (vegetatively) expanded before it is sold to individual customers years later. After the second year, samples of tubers are taken for density measurements and preliminary fry tests to determine the suitability of the tubers for commercial use. A multitude of tubers of plants which have survived the selection process to this point are then planted in the third year for a more comprehensive series of fry tests and density determinations. At the fourth-year stage of development, a diminishing number of surviving selections is grown in ever expanding numbers and plants thereof are subjected to field trials in several stages to determine their adaptability to different growing conditions. Eventually, the varieties having superior agronomical qualities are transferred to other farms and the "seed" (in the form of tubers) is increased to commercial scale. Since one "seed" tuber may generate between 6 and 20 harvested tubers this up-scaling process may take years before sufficient "seed" is produced. Generally, by this time, eight or more years of planting, harvesting and testing have been invested in attempting to develop a new and improved potato cultivar.

To reduce inbreeding depression a breeder may introduce new genes from a genetically more remote parent such as from wild and primitive species with ploidy levels ranging from diploid to hexaploid. However, when two genetically unrelated potato plants are crossed, the level of heterozygosity may be increased but simultaneously more deleterious genes are also introduced. As a consequence, a breeder will typically make additional crosses with more commercial germplasm to enrich the population for favourable alleles. All together such a multiple crossing breeding programme may take dozens of years as the selection of the favourable genotypes in each generation may already take five years. Therefore, potato breeding is currently a predominantly empirical exercise, strongly characterised by trial and error.

Potatoes and their related wild species (tuber-bearing *Solanum* species) are mostly outbreeding because self fertilisation is hampered by a gametophytic self-incompatibility system. Self-incompatibility (SI) is a general name for several genetic mechanisms in angiosperms, which prevent self-fertilization and inbreeding. In plants with SI, when a pollen grain produced in a plant reaches a stigma of the same plant or another plant with a similar genotype, the process of pollen germination, pollen tube growth, ovule fertilization, and embryo development is halted at one of its stages, and consequently no seeds are produced. Self-incompatibility is not found in tetraploid potatoes.

The provision of such self-compatible clones may facilitate the generation of selfed progenies of potato, and hence the production of (highly) homozygous potato lines. This could provide a great opportunity to the development of homozygous elite breeding lines in potato. However, to date, the development of homozygous elite lines with genetically fixed agronomically desirable traits, that would enable the production of genetically uniform hybrid potato seed, has been unsuccessful.

The provision of homozygous elite lines is hampered by unknown causes. Selfing of an occasionally encountered self-compatible clone results in a slower decrease of heterozygosity than theoretically expected. The slower decline of heterozygosity may be the result of unintentional but unavoidable selection during selfing for the heterozygous plants in the offspring that exhibit higher vigour, fertility and seed germination. It is implied that, as the heterozygosity is reduced by selfing, the fertility and vigour are also reduced and the plants may become weak and completely sterile. The situation is perhaps worsened by the establishment of homozygous configurations of recessive deleterious genes. This phenomenon which is generally referred to as inbreeding depression has greatly hampered the development for homozygous potato lines and hence the production of uniform hybrid potato seed.

There is a large prejudice against the production of homozygous breeding lines in potato due to inbreeding depression. Uijtewaal et al. (Euphytica 36 (1987) 745-753) indicated that owing to sterility problems, homozygous potato clones would be of little importance for practical breeding. Developing homozygous inbred lines was considered, but rendered impossible in potato (Umaerus, 1987, Proceeding of the 10th Triennial Conference of the European Association of Potato Research, Aalborg, Denmark, pp 72–103 as cited in Almekinders et al. 2009 Potato Research 52:275–293). Routes involving doubling of haploids have long been presumed as promising. Nontheless, up to the present day the ruling opinion is that inbreeding depression in diploid potato is too strong to ever result in vigorous homozygous plants.

Birhman and Hosaka (Genome 43: 495–502 (2000)) have suggested the possibility to utilize the *Sli* gene derived from *S. chacoense* for development of highly homozygous true potato seed (TPS) lines and heterosis breeding of the potato. However, to date, no homozygous lines with good agronomic traits such as good tuber yield, have ever been reported from this proposed line of research. Rather, those homozygotes that have been produced do not exhibit any agronomically relevant tuber yield.

Rommens in 2010 (Genetic modification of Plants, Kempen & Jung , eds, In: Biotechnology in Agriculture and Forestry 64(1): 61-77 (2010)), advocates the route of genetic transformation, due to the fact that efforts to improve the yield and quality of this crop are hampered by inbreeding depression.

In short, production of true breeding lines of potato is considered impossible. Due to this fact, potato breeding cannot escape the traditional schemes based on crossing of tetraploid heterozygotes. As a result, the challenge is considered formidable to combine various utilization traits (relating to fresh and processing uses), resistances to pathogens and pests, and numerous other relevant agronomic traits with improvements in yield into a commercially acceptable cultivar (Douches et al. 1999, Crop Science 36(6):1544-1552).

It is an objective of the present invention to provide means and methods for the production of elite breeding lines of potato and for the production of uniform hybrid potato seed from which plants can be grown that exhibit agronomically relevant tuber yield.

SUMMARY OF THE INVENTION

The present inventors have attempted to achieve homozygous parental breeding lines for generative propagation of potatoes via a number of routes, none of which were successful. These unsuccessful routes included androgenesis, gynogenesis, doubling of haploids and continued selfings. Only by repeated selfing of a self-compatible plant comprising the *Sli* gene derived from *S. chacoense* and the use of did the inventors arrive at the product of the present invention. The present invention now provides in a first aspect a diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant. Plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant are in general terms also referred to herein as being vigorous.

In a preferred embodiment, said line in addition to said tuber yield comprises at least one, more preferably at least 2, 3, 4, 5 or 6 (additional) agronomically desirable traits. Preferably, said agronomically desirable traits are selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to

resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, and cold sweetening resistance.

In another preferred embodiment of said potato line of the invention, plants of said line when mature attain an average fresh weight of the foliage and shoots of at least 500 grams per plant. Plants attaining an average fresh weight of the foliage and shoots of at least 500 grams per plant are in general terms also referred to herein as being vigorous.

In another aspect, the present invention provides a plant of a line according to the invention as described above. Preferably, said plant is diploid, fertile, self-compatible and essentially homozygous and has an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant.

In another aspect, the present invention provides a seed of a plant as described above. Said seed may be homozygous (i.e. represent a selfed seed), but may also be heterozygous for the alleles where the parents differ. In one embodiment, said seed is preferably a uniform hybrid potato seed. Such uniform hybrid potato seed being the result of a cross between two homozygous, self-compatible, fertile and vigorous plants of a line according to the invention as described above, preferably of two different lines, wherein the lines contain at least 20 % contrasting homozygous loci as determinable by molecular marker analysis.

In another aspect, the present invention provides a method for producing a diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, comprising:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, self-compatible, fertile and essentially homozygous potato line;

(b) providing a second potato plant, wherein said second potato plant is a plant of a diploid or tetraploid potato line which may have any level of homozygosity, said line of said second potato plant comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant;

(c) cross pollinating said first and second potato plant to provide seeds and collecting said seeds to thereby provide an offspring generation in the form of a hybrid offspring seed;

(d) growing said hybrid offspring seed into a population of hybrid offspring potato plant and selecting from said population plants producing tubers at a yield expressed in grams of fresh weight of at least 200 grams per plant, and

(e) selfing said hybrid offspring potato plant or backcrossing said hybrid offspring potato plant to a potato plant of said line of said first potato plant, for between 1 to 8 selfings or backcrosses, to thereby provide a diploid, self-compatible and essentially homozygous potato breeding line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant.

A first potato plant in a method as described herein is preferably a plant of a first diploid, self-compatible, fertile and essentially homozygous potato line, wherein the self-compatibility is conditioned by the presence of a dominant allele of an S-locus (self-incompatibility locus) inhibitor gene (Sli) that is nonallelic to the S locus as described in Hosaka and Hanneman 1998 Euphytica, 99: 191–197.

The above method, when performed on a sufficiently large scale (at least more than 10.000 plants) and performed with the use of a suitable second potato plant, which is preferably a diploid potato clone that is essentially not homozygous, said line of said second potato plant comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, preferably a line such as IVPAA-096-18 (D2) as described in the Examples herein below, results in the production of an essentially homozygous, fertile, vigorous and self-compatible breeding line, wherein said vigour is in particular represented by an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant. The provision of such a breeding line is a crucial step in the provision of genetically homozygous true potato seed (TPS).

The first and second breeding line can subsequently be used in the production of the TPS when both the first and second breeding line comprise agronomically desirable characteristics, have good general combining abilities, and preferably have at least 20% contrasting homozygous loci as determinable by molecular marker analysis.

In a preferred embodiment of said method for producing a diploid, fertile, self-compatible and essentially homozygous potato line according to the invention, said steps a-e are repeated wherein step b comprises the provision of third (or further) potato plant wherein said third potato plant is a plant of a diploid or tetraploid potato line which may have any level of homozygosity, wherein said third potato plant is a donor for an additional agronomically desirable trait. Preferably, said trait is selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, cold tolerance, drought tolerance, wet

tolerance, tolerance to dry and wet rot, salinity tolerance, and cold sweetening resistance, or any other agronomically beneficial trait or desirable characteristic as defined herein.

The second or third potato plant as described above may suitably be a diploid potato clone selected from the plants D1-D21 as listed in Table 6 of Example 3 below. The skilled person will understand that other diploid potato clones may also be used. Highly preferred is the use of potato clone IVPAA-096-18 (D2) which is available from Laboratory of Plant Breeding (IVP) (Dr. Ir Ronald B.C. Hutten), Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.

Proper (commercial) sources of tetraploid cultivars are provided through the European Cultivated Potato Database (ECPD) at http://www.europotato.org/menu.php and/or the Potato Pedigree Database of the expertise group Plant Breeding (a cooperation between the Laboratory of Plant breeding of Wageningen UR, the Netherlands) and the business unit Biodiversity and breeding of Plant Research International (PRI), Wageningen, the Netherlands at http://www.plantbreeding.wur.nl/potatopedigree/.

In another preferred embodiment of said method, said first potato plant is a plant of potato line NCIMB 41663, NCIMB 41664, NCIMB 41665, or NCIMB 41765, representative seeds of said line having been deposited with the NCIMB, Aberdeen, Scotland.

In yet another preferred embodiment of said method, said second potato plant is a plant of a second diploid, fertile, self-compatible and essentially homozygous potato line.

In yet a further preferred embodiment of said method, said second potato plant is a plant of a second diploid, fertile, self-incompatible potato line.

The method for producing a diploid potato breeding line may further comprise the steps of enhancing the breeding value of the diploid potato breeding line by intercrossing, selfing and backcrossing while continuous selecting for agronomically desirable traits. Experimental crosses are done to evaluate general combining abilities (intercrossability).

In another aspect, the present invention provides a diploid potato breeding line, more preferably an elite line, obtainable by the method of the present invention as described above.

In another aspect, the present invention provides a hybrid potato seed obtainable by crossing plants of a first and second diploid potato breeding line according to the present invention as described above, wherein said plants of said first and second

diploid potato breeding line contain at least 20 % contrasting homozygous loci as determinable by molecular marker analysis.

In another aspect, the present invention provides a method for producing a uniform hybrid potato seed, comprising:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, fertile, self-compatible and essentially homozygous potato line,

(b) providing a second potato plant, wherein said second potato plant is a plant of a second diploid, fertile, self-compatible and essentially homozygous potato line, wherein said first and second plant contain at least 20 % contrasting homozygous loci as determinable by molecular marker analysis, and

(c) cross pollinating said first and second potato plant and allowing one of said parent plants to produce berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed.

In a preferred embodiment of a method for producing hybrid seed, said first and second potato plant are from potato lines comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, preferably wherein said first and second potato plant are plants having a tuber yield of at least 200 grams fresh weight per plant.

In another preferred embodiment of said method of producing hybrid seed, at least one of said first and second potato plants comprises at least one agronomically desirable trait, preferably selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, and cold sweetening resistance.

In another preferred embodiment of a method for producing hybrid seed according to the present invention, said first potato line is a diploid, self-compatible and essentially homozygous offspring potato plant comprising the *Sli*-gene, preferably wherein said first potato plant is a plant of potato lines NCIMB 41663, NCIMB 41664, NCIMB 41665, or NCIMB 41765, representative seeds of said lines having been deposited with the NCIMB, Aberdeen, Scotland, or a diploid, self-compatible and essentially homozygous offspring potato plant thereof.

In another preferred embodiment of a method for producing hybrid seed according to the present invention, said second potato plant is a plant of a second diploid, self-compatible and essentially homozygous potato line preferably comprising at least two

agronomically desirable traits selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, and cold sweetening resistance.

In another aspect, the present invention provides a seed of potato lines NCIMB 41663, NCIMB 41664, NCIMB 41665, or NCIMB 41765, representative seeds of said line having been deposited with the NCIMB, Aberdeen, Scotland.

In another aspect, the present invention provides a seed produced by the method for producing a uniform hybrid potato seed as disclosed herein.

In another aspect, the present invention provides a hybrid potato plant, or part thereof, produced by growing the seed of the present invention.

In another aspect, the present invention provides pollen or an ovule of the hybrid potato plant, or part thereof, of the present invention.

In another aspect, the present invention provides a tuber from the hybrid potato plant of the present invention.

In another aspect, the present invention provides a potato plant having all of the physiological and morphological characteristics of the hybrid potato plant produced by growing the seed of the present invention.

In another aspect, the present invention provides a tissue culture of the hybrid potato plants of the present invention.

In another aspect, the present invention provides a potato plant regenerated from the tissue culture of the present invention, wherein said regenerated potato plant has all the physiological and morphological characteristics of the hybrid potato plant produced by growing the seed of the present invention.

In another aspect, the present invention provides a method for producing a hybrid potato seed wherein the method comprises crossing a first potato plant with a

second potato plant and harvesting the resultant hybrid potato seed, wherein said first and/or second parent potato plant is a diploid, fertile, self-compatible and essentially homozygous plant according to the present invention that has an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant.

In another aspect, the present invention provides a method of producing a hybrid potato plant, said method comprising producing a seed according to a method of the present invention and growing said seed into a potato plant.

In another aspect, the present invention provides a potato plant produced by the method of the invention, or a tuber thereof.

In another aspect, the present invention provides a method set of at least 4 intercrossable homozygous diploid, fertile, self-compatible and essentially homozygous potato lines comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant according to the present invention, wherein each of said lines differ from another line in said set by having at least 20 % contrasting homozygous loci as determinable by molecular genetic marker analysis.

The invention also provides as an embodiment of methods of the invention as described above methods comprising the steps of:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, self compatible and essentially homozygous potato line carrying chromosomes with alleles for agronomically desirable traits that are to be expressed in an offspring generations,

(b) providing a second potato plant, wherein said second potato plant is a plant of a second diploid, self compatible and essentially homozygous potato line carrying chromosomes with alleles for agronomically desirable traits that are to be expressed in an offspring generations and that may be different form the traits in the said first plant, and

(c) cross pollinating said first and second potato plant such that one of said parent plants produces berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed carrying combinations of chromosomes with said alleles.

Thus, in an alternative embodiment the plant of the first potato line may carry chromosomes with alleles for agronomically desirable traits that are to be expressed in an

offspring generations, in which case the agronomically desirable trait in the plant of the second potato line may be different from the traits in the said first plant.

In another aspect, the present invention provides a method of producing a herbicide resistant, nematode resistant, insect resistant and/or disease resistant potato plant, said method comprising producing a seed according to a method of the invention described above by crossing parental lines that are herbicide resistant, nematode resistant, insect resistant and/or disease resistant and growing said seed a potato plant.

In another aspect, the present invention provides a herbicide resistant, nematode resistant, insect resistant and/or disease resistant potato plant produced by the method of the invention as described above.

In yet another aspect, the present invention provides a method for preventing transmittal of virus infections between potato generations, comprising:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, self-compatible and essentially homozygous potato line,

(b) providing a second potato plant, wherein said second potato plant is a plant of a potato line that may have any level of ploidy and/or any level of homozygosity, said second potato plant carrying a chromosome with an allele for a agronomically desirable trait that is to be expressed in an offspring generation;

(c) cross pollinating said first and second potato plant such that one of said parent plants produces berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed carrying a chromosome with said allele;

(d) growing said hybrid offspring seed into a hybrid offspring potato plant carrying a chromosome with said allele, and

(e1) harvesting tubers from said hybrid offspring potato plant to provide tubers expressing said trait, or

(e2) selfing said hybrid offspring potato plant or backcrossing said hybrid offspring potato plant to a potato plant of said line of said first potato plant, thereby providing further offspring plants carrying a chromosome with said allele and wherein said offspring plants are increasingly homozygous following each backcrossing or selfing step.

In another aspect, the present invention provides a diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, said plant being obtainable by:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, fertile, self-compatible and essentially homozygous potato line,

(b) providing a second potato plant, wherein said second potato plant is a plant of a diploid potato line having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant,

(c) cross pollinating said first and second potato plant and allowing one of said parent plants to produce berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed,

(d) growing said hybrid offspring seed into a hybrid offspring potato plant,

(e) backcrossing said hybrid offspring potato plant to a potato plant of said line of said first potato plant, and selecting from the offspring plants thus generated a plant that is diploid, self-compatible and vigorous in that it produces tubers at a yield expressed in grams of fresh weight of at least 200 grams per plant, and

(f) selfing said diploid, self-compatible and vigorous plant for between 1 to 8 generations while selecting for self-compatibility and vigour as defined in (e).

In another aspect, the present invention provides a hybrid potato seed which seed, when sown produces a diploid, fertile, self-compatible and essentially heterozygous potato plant having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, said plant being obtainable by:

(a) providing a first diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant,

(b) providing a second diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, wherein said first and second plant contain at least 20 % contrasting homozygous loci as determinable by molecular marker analysis, and

(c) cross pollinating said first and second potato plant and allowing at least one of said parent plants to produce berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed.

The steps of selecting or providing plants that are homozygous, or the step of selecting or providing plants having at least 20% contrasting loci as defined herein above, may comprise marker assisted selection using the molecular genetic markers as provided herein.

DESCRIPTION OF THE DRAWINGS

~~Figure~~ FIG. 1 shows a schematic presentation of a development scheme for the development of hybrid potato cultivars using the methods and plants of the present invention, wherein the various steps in obtaining commercial potato hybrids are indicated:

Box 0, Potato germplasm: The origin comprises in principle all potato genotypes within the cultivated potato species (*Solanum tuberosum*) and related crossable species;

Box 1, Self-compatible diploid potato: Cultivated potato (*Solanum tuberosum*) is usually self incompatible. Self-compatibility is introduced from related species (see for instance Hermsen, J.G.Th. 1978, Euphytica 27, 1-11);

Box 2, Homozygous fertile self-compatible diploid potato: Homozygous diploids can be obtained by doubling haploids (Uijtewaal et al., 1987, Theor. Appl. Genet. 73, 751-78) by anther culture (Jacobsen and Sopory, 1977, Theor. Appl. Genet. 52, 119-123), by ovule culture or by repeated selfings (Phumichai & Hosaka, 2006, Euphytica 149, 251-258). The example of the latter is illustrated herein;

Box 3, Vigorous fertile homozygous self-compatible diploid potato: The vigour is enhanced by crossing with other diploid potato, selfing and backcrossing while selecting for higher vigour;

Box 4, Diploid potato breeding Lines: Agronomically desirable traits are introduced by crossing with other diploid potato, selfing and backcrossing while selecting for these traits;

Box 5, Elite diploid potato breeding Lines: The breeding value is further enhanced by intercrossing, selfing and backcrossing while continuous selecting for agronomically desirable traits. Experimental crosses are done to evaluate general combining abilities;

Box 6 Potato hybrid cultivars: Good combining breeding lines are crossed. The seeds obtained are hybrid true potato seeds that are uniform.

~~Figure~~ FIG. 2 shows a schematic presentation of the pedigrees of the populations, deposits and plants as described in the Examples, starting from a cross between a diploid, essentially homozygous and self-compatible potato plant ("H") and three different diploid clones (D1, D2, and D3) that are essentially non-homozygous and self-incompatible and that act as donor lines of one or more desirable agronomic traits. The production of an array of essentially homozygous lines from backcross lines to fully selfed clones, each having one or more desirable traits derived from the donor lines provides a set of elite breeding lines for the production of an array of hybrid potato cultivars in the form of hybrid seed. ⊗ Refers to selfings. As can be seen, and as detailed in the Examples, only

donor line D2 resulted in the production of a large number of self-compatible plants that would show vigour as defined herein. Vigorous plants could be obtained from the other crossings as well, albeit at a much lower frequency (0.1-1%).

~~Figure 3~~ FIG. 3A - FIG. 3F display[[s]] the ultra-dense genetic linkage map of the genome of diploid breeding clone RH89-039-16. The number on the left of the linkage group indicates the cumulative number of recombination events counted from the top. The number of AFLP markers in each bin is represented by shades of grey according to the figure legend. Putative centromere positions are indicated with "Cen" alongside the chromosome (van Os et al., 2006 Genetics 173(2):1075-1087). The numbers on the right of the linkage group indicate the approximate location of the corresponding PotSNPmarker as used herein, wherein reference is made to Table 5B in Example 3, below, for marker annotations. The figure clearly indicates the distribution of informative markers over the potato genome of this clone. A similar distribution was observed for these markers with another clone used herein, clone SH83-92-488.

~~Figure 4~~FIG.4A - FIG. 4L show[[s]] marker data of 100 markers over 20 different diploid potato genotypes, representing the diploid potato breeding germplasm (Example 2).

~~Figure 5~~FIG. 5A-1 - FIG. 5F - 4 show[[s]] the result of SNP marker analysis as described in Example 3 on a set of six F3 populations and their respective parents.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

The term "potato" is used herein to refer to material that is essentially of species *Solanum tuberosum*, but may include introgression segments of other tuber bearing *Solanum* species such as *Solanum chacoense, Solanum phureja, Solanum andigena, Solanum demissum.*

The term "potato plant" is used herein to refer to a seedling or mature plant as grown from cell culture or seed. Persons of ordinary skill in the art will recognize that when the term "potato plant" is used in the context of the present invention, this also includes derivative varieties that retain the essential distinguishing characteristics of for instance potato varieties that are subject of the present invention, such as a Single Gene Converted plant of that variety or a transgenic derivative having one or more value-added genes incorporated therein (such as herbicide or pest resistance). Backcrossing methods can be used with the present invention to improve or introduce a characteristic into the variety.

The term "crossing" as used herein refers to the fertilization of female plants (or gametes) by male plants (or gametes). The term "gamete" refers to the haploid reproductive cell (egg or sperm) produced in plants by mitosis from a gametophyte and involved in sexual reproduction, during which two gametes of opposite sex fuse to form a diploid zygote. The term generally includes reference to a pollen (including the sperm cell) and an ovule (including the ovum). "Crossing" therefore generally refers to the fertilization of ovules of one individual with pollen from another individual, whereas "selfing" refers to the fertilization of ovules of an individual with pollen from the same individual. Crossing is widely used in plant breeding and results in a mix of genomic information between the two plants crossed one chromosome from the mother and one chromosome from the father. This will result in a new combination of genetically inherited traits. Usually, the progeny of a crossing is designated as: “F1”. If the F1 is not uniform (segregates) it is usually designated as “F1 population”. “Selfing” of a homozygous plant will usually result in a genetic identical plant since there is no genetic variation. “Selfing” of an F1 will result in an offspring that segregates for all traits that have heterozygotic loci in the F1. Such offspring is designated: “F2” or “F2 population”

When referring to “crossing” in the context of achieving the introgression of a genomic region or segment, the skilled person will understand that in order to achieve the introgression of only a part of a chromosome of one plant into the chromosome of another plant, it is required that random portions of the genomes of both parental lines will be recombined during the cross due to the occurrence of crossing-over events in the production of the gametes in the parent lines. Therefore, the genomes of both parents must be combined in a single cell by a cross, where after the production of gametes from said cell and their fusion in fertilization will result in an introgression event.

The term “intercrossable”, as used herein, refers to the ability to yield progeny plants after making crosses between parent plants.

As used herein, the terms "introgressing", "introgress" and "introgressed" refer to both a natural and artificial process whereby individual genes or entire chromosomes are moved from one individual, species, variety or cultivar into the genome of another individual, species, variety or cultivar, by crossing those individuals, species, varieties or cultivars. In plant breeding, the process usually involves selfing or backcrossing to the recurrent parent to provide for an increasingly homozygous plant having essentially the characteristics of the recurrent parent in addition to the introgressed gene or trait.

The term “introgression” refers to the result of an introgression event.

The term ”backcross” refers to the result of a “backcrossing” process wherein the plant resulting from a cross between two parental lines is (repeatedly) crossed with one

of its parental lines, wherein the parental line used in the backcross is referred to as the recurrent parent. Repeated backcrossing results in replacement of genome fragments of the donor parent with those of the recurrent. The offspring of a backcross is designated "BCx" or "BCx population", where "x" stands for the number of backcrosses.

The term "backcrossing" as used herein refers to the repeated crossing of a hybrid progeny back to the recurrent parents. The parental potato plant which contributes the gene for the desired characteristic is termed the nonrecurrent or donor parent. This terminology refers to the fact that the nonrecurrent parent is used one time in the backcross protocol and therefore does not recur. The parental potato plant to which the gene or genes from the nonrecurrent parent are transferred is known as the recurrent parent as it is used for several rounds in the backcrossing protocol. In a typical backcross protocol, the original variety of interest (recurrent parent) is crossed to a second variety (nonrecurrent parent) that carries the single gene of interest to be transferred. The resulting progeny from this cross are then crossed again to the recurrent parent and the process is repeated until a potato plant is obtained wherein essentially all of the desired morphological and physiological characteristics of the recurrent parent are recovered in the converted plant, in addition to the single gene or a limited number of genes transferred from the nonrecurrent parent.

The selection of a suitable recurrent parent is an important step for a successful backcrossing procedure. The goal of a backcross protocol is to alter or substitute a single trait or characteristic in the original variety. To accomplish this, a single gene of the recurrent variety is modified, substituted or supplemented with the desired gene from the nonrecurrent parent, while retaining essentially all of the rest of the desired genes, and therefore the desired physiological and morphological constitution of the original variety. The choice of the particular nonrecurrent parent will depend on the purpose of the backcross. One of the major purposes is to add some commercially desirable, agronomically important traits to the recurrent parent. The exact backcrossing protocol will depend on the characteristic or trait being altered or added to determine an appropriate testing protocol. Although backcrossing methods are simplified when the characteristic being transferred is a dominant allele, a recessive allele may also be transferred. In this instance, it may be necessary to introduce a test of the progeny to determine if the desired characteristic has been successfully transferred. Preferably, such genes are monitored by diagnostic molecular markers.

Likewise, transgenes can be introduced into the plant using any of a variety of established recombinant methods well-known to persons skilled in the art.

Many single gene traits have been identified that are not regularly selected for in the development of a new variety but that can be improved by backcrossing and genetic engineering techniques. Single gene traits may or may not be transgenic, examples of these traits include but are not limited to: herbicide resistance; resistance to bacterial, fungal or viral disease; insect resistance; uniformity or increase in concentration of starch and other carbohydrates; enhanced nutritional quality; decrease in tendency of tuber to bruise; and decrease in the rate of starch conversion to sugars.

The term “selfing” refers to the process of self-fertilization wherein an individual is pollinated or fertilized with its own pollen. Repeated selfing eventually results in homozygous offspring.

A “line”, as used herein, refers to a population of plants derived from a single cross, backcross or selfing. The individual offspring plants are not necessarily identical to one another. It is possible that individual offspring plants are not vigorous, fertile or self compatible due to natural variability. However, it is foreseen that suitable plants that are vigorous, fertile and self compatible can be easily identified in a line and used for additional breeding purpose.

As used herein, the term “allele(s)” means any of one or more alternative forms of a gene, all of which alleles relate to at least one trait or characteristic. In a diploid cell or organism, the two copies of a gene occupy corresponding loci on a pair of homologous chromosomes. Each copy may be a distinct allele.

A “gene” is defined herein as a hereditary unit (often indicated by a sequence of DNA) that occupies a specific location on a chromosome and that contains the genetic instruction for a contribution to potential phenotypic characteristics or trait in a plant.

A “locus” is defined herein as the position that a given gene occupies on a chromosome of a given plant species.

As used herein, the term "homozygous" means a genetic condition existing when identical alleles reside at corresponding loci on homologous chromosomes.

The term “essentially homozygous” refers to a level of homozygosity of at least 25%, preferably at least 50%, more preferably at least 75%, still more preferably at least 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% homozygosity when testing 100, preferably 1000, more preferably at least 10.000 loci.

Homozygosity levels are average values for the population, and refer preferably to those loci wherein the parents differ.

As used herein, the term "heterozygous" means a genetic condition existing when different alleles reside at corresponding loci on homologous chromosomes.

The term “recombination” or “recombine” refers to the exchange of information between two homologous chromosomes during meiosis. In a “recombinant” plant, DNA that is originally present on a specific location within the chromosome, e.g. linked to a gene/locus, is exchanged for DNA from another plant (i.e. maternal for paternal or *vice versa*). In order to exchange only the required material, and maintain the valuable original information on the chromosome as much as possible, two flanking crossover or recombination events will usually be required. In a double recombinant this exchange has taken place on both sides of a gene/locus. one way to find such a double recombinant, is to screen a population of F2-plants. This population has to be large, since double recombination occurs in only a limited frequency. Alternatively, double recombinants within a genetic unit can be the result of subsequent backcrossing. The frequency of double recombination is the product of the frequencies of the single recombinants. (E.g. a recombinant in a 10 cM area can be found with a frequency of 10%, double recombinants are found with a frequency of 10% x 10% = 1 %).

As used herein, the term "progeny" means (a) genetic descendant(s) or offspring.

As used herein, the term "population" means a genetically heterogeneous collection of plants sharing a common genetic derivation.

A "recombination event" refers to a mitotic or meiotic crossing-over event, including a GMO event.

As used herein, the term "hybrid" means any offspring of a cross between two genetically unlike individuals, more preferably the term refers to the cross between two (elite or inbred) breeding lines which will not reproduce true to the parent from seed.

The term “segregate”, as used herein, refers to the separation of paired alleles during meiosis so that members of each pair of alleles appear in different gametes. The term includes reference to the result of this genetic phenomenon wherein the offspring population of a crossing in which at least one of the parents is heterozygous for an allelic gene is non-uniform with respect to phenotypic trait conferred by said gene.

The term “breeding line”, as used herein, refers to a line of a cultivated potato having commercially valuable or agronomically desirable characteristics, as opposed to wild varieties or landraces. The term includes reference to an elite breeding line or elite line, which represents an essentially homozygous, usually inbred, line of plants used to produce commercial F_1 hybrids. An elite breeding line is obtained by breeding and selection for superior agronomic performance comprising a multitude of agronomically desirable traits. An elite plant is any plant from an elite line. Superior agronomic performance refers to a desired combination of agronomically desirable traits as defined

herein, wherein it is desirable that the majority, preferably all of the agronomically desirable traits are improved in the elite breeding line as compared to a non-elite breeding line. Elite breeding lines are essentially homozygous and are preferably inbred lines.

The term "elite line", as used herein, refers to any line that has resulted from breeding and selection for superior agronomic performance. An elite line preferably is a line that has multiple, preferably at least 3, 4 5, 6 or more (genes for) desirable agronomic traits as defined herein.

The terms "cultivar" and “variety” are used interchangeable herein and denote a plant with has deliberately been developed by breeding, cq crossing and selection, for the purpose of being commercialized, cq used by farmers and growers, to produce agricultural products for own sonsumption or for commercialization (fresh consumption, processing, feed, etc). The term “breeding germplasm” denotes a plnat having a biological status other than a "wild" status, which "wild" status indicates the original non-cultivated, or natural state of a plant or accession.

The term "breeding germplasm" includes, but is not limited to, semi-natural, semi-wild, weedy, traditional cultivar, landrace, breeding material, research material, breeder's line, synthetic population, hybrid, founder stock/base population, inbred line (parent of hybrid cultivar), segregating population, mutant/genetic stock, market class and advanced/improved cultivar. Examples of cultivars include such cultivated varieties as Bintje, Russet Burbank, Eigenheimer, and Nicola.

As used herein, the terms "purebred", "pure inbred" or “inbred” are interchangeable and refer to a substantially homozygous plant or plant line obtained by repeated selfing and-or backcrossing.

As used herein, the term “molecular genetic marker” or short “marker” refers to an indicator that is used in methods for visualizing differences in characteristics of nucleic acid sequences. Examples of such indicators are restriction fragment length polymorphism (RFLP) markers, amplified fragment length polymorphism (AFLP) markers, single nucleotide polymorphisms (SNPs), insertion/deletion (INDEL) mutations, microsatellite markers (SSRs), sequence-characterized amplified regions (SCARs), cleaved amplified polymorphic sequence (CAPS) markers or isozyme markers or combinations of the markers described herein which defines a specific genetic and chromosomal location.

As used herein, the term "plant part" indicates a part of the potato plant, including organelles, single cells and cell tissues such as plant cells that are intact in plants, cell clumps and tissue cultures from which potato plants can be regenerated. Examples of plant parts include, but are not limited to, single cells and tissues from pollen, ovules, leaves, embryos, roots, root tips, tubers, anthers, flowers, fruits, stems shoots, and seeds; as

well as pollen, ovules, leaves, embryos, roots, root tips, anthers, flowers, fruits, stems, shoots, scions, rootstocks, seeds, tubers, protoplasts, calli, and the like.

Unless expressly stated otherwise, the term "seed", as used throughout this specification, refers to the body from which a new plant develops (or kernel in some plants) containing the small embryonic plant enclosed in a seed coat covering, usually together with some stored food. This seed, referred to as botanical or "true" seed is the product of the ripened ovule of gymnosperm and angiosperm plants which occurs after fertilization and some growth within the mother plant.

As used herein the terms "vigour" and "vigorous" refer to the relative amount of above-ground or below-ground tissues of a plant, which relative amount may be more or less independent of one another.

The relative amount of above-ground tissues can be expressed as visual observations of the amount of foliage, in terms of plant height, number of stems, number of leaves assessed on ordinal scales, and/or in terms of fresh weight or dry matter. This trait has relevance during the first period of the plant growth cycle with an average duration of 100 days of cultivation (range 75 – 130 days) under normal growth conditions that prevail in North Western Europe during the season from May trough to August. Under these conditions, the plants of the present invention attain a weight of the foliage and shoots (above-ground plant) expressed in grams of fresh or dry weight which is at least 50%, more preferably at least 60%, 70%, 80%, 90% or more of the (essentially heterozygous) diploid breeding clone RH89-039-16, which – in turn - has 68% (range 44 – 86%) of the tuber yield of tetraploid potato varieties based on nine years of testing. Clone RH89-039-16 is available for reference purpose from Dr. Ir Ronald B.C. Hutten, Laboratory of Plant Breeding, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.

Alternatively, or in addition, the term vigour may be used in connection with a certain minimal tuber yield. The term "tuber yield", as used herein, will generally refer to a tuber yield expressed in grams of fresh weight which at harvest in plants according to the present invention is at least 30-50%, more preferably at least 60%, 70%, 80%, 90% or more of the (essentially heterozygous) diploid breeding clone RH89-039-16, which – in turn - has 68% (range 44 – 86%) of the tuber yield of tetraploid potato varieties based on nine years of field testing under conditions of a 100 days cultivation period with normal growth conditions that prevail in North Western Europe during the season from May trough to August. As indicated above, clone RH89-039-16 is available for reference purpose from Dr. Ir Ronald B.C. Hutten, Laboratory of Plant Breeding, Wageningen University.

Tuber yield, unless expressly stated otherwise, is based on the tubers formed from a seedling plant, in contrast to the tubers formed from a tuber-grown plant – which yield may be 50-200% more than the yield of a seedling.

Alternatively, or in addition, the term vigour may refer to a tuber yield expressed in grams of fresh or dry weight which at harvest is at least 30-50%, more preferably at least 60%, 70%, 80%, 90% or more of the diploid breeding clone RH89-039-16. This means that the tuber yield expressed in grams of fresh weight may (in plants of the present invention, reach levels of at least 200, more preferably at least 300, 400 or 500 grams per plant grown from seed in clay soil under normal North Western European summer conditions, and even 400, 500, 600, 700, 800 or more, such as 900 or 1000 grams of fresh tuber weight or more per plant when said plant is raised from tubers and grown in clay soil under normal North Western European summer conditions.

The term "a line comprising plants", wherein said plants are indicated as having a certain characteristic, such as tuber yield, should preferably be understood as referring to a line essentially consisting of plants having the said trait, while allowing for some biological variation.

The term "diploid" as used herein refers to a plant wherein each vegetative cell contains two sets of chromosomes (2x=2n, wherein n is the number of chromosomes). One set of chromosomes is donated from each parent.

The term "tetraploid" as used herein refers to a plant wherein each vegetative cell contains four sets of chromosomes (2x=4n).

The term "nematode resistance" as used herein refers to a plant in which a functional resistance gene has been introgressed that prevents the multiplication of at least one nematode population or isolate.

The term "disease resistance" as used herein refers to the ability to show more than 50 % reduction of the amount of diseased leaf surface or tuber volume, or the amount of multiplication of an insect or pathogenic microorganism including but not limited to *Streptomyces* spp, *Rhizoctonia*, Silver scurf and, *Phytophthora infestans*,

The term "herbicide tolerance" as used herein refers to a plant which shows less than 50% damage of the foliage than the usual damage upon the application of a specified dosage of herbicide.

The term "cold tolerance" as used herein refers to the average amount of leaf surface of a plant which shows frost damage, which damage in cold tolerant plants is less than the average damage observed at a temp of minus 3 °C for relatively cold sensitive reference varieties such as e.g. Caribe and/or Kennebec.

The term "drought tolerance" as used herein refers to a plant which shows less than average damage at limiting water conditions as compared to relatively drought sensitive reference varieties such as Caribe and/or Carlton.

The term "flooding tolerance" as used herein refers to a 50% lower proportion of affected tubers (anaerobic degradation) relative to a plant which shows average damage at flooding

The term "tolerance to wet rot" as used herein refers to a plant with the slightest level of resistance to *Erwinia* species (currently termed *Pectobacterium* spp.).

The term "tolerance to dry rot" as used herein refers to a plant with the slightest level of resistance to *Fusarium* species.

The term "salinity tolerance" as used herein refers to a plant which shows less damage than average at salinity conditions.

The term "growth rate" as used herein refers to the increase of plant biomass per unit of time.

The term "tuber development defects" (e.g. misshapen or damaged tubers)" as used herein refers to a plant which shows visual as well as internal malformations of tubers during plant development and/or tuber harvest.

The term "tuber yield" as used herein refers to the total weight of tubers of a plant or of a population of plants generally expressed in grams of fresh weight.

The term "tuber size" as used herein refers to the absolute width, height and length of a tuber. Beneficial tuber sizes include plant- average tuber sizes of about 80-160 grams per tuber. Mean tuber number for commercially valuable plants are about 8-12 tubers per plant.

The term "tuber skin colour" as used herein refers to the colour of the skin of a tuber after harvest as the result of anthocyanin accumulation in tuber skin tissues.

The term "eye depth" as used herein refers to the relative distance between the skin surface and the shoot primordium of a tuber.

The term "tuber shape" as used herein refers to the length / width ratio, to indicate the continuous variation from round, oval to long tuber shaps, as well as the height/width ratio, to indicate the continuous variation from cylindrical to the amount of flatness of a tuber.

The term "tuber flesh colour" as used herein refers to the colour of the interior of the tuber flesh after harvest as the result of the absence or presence of carotenoid compounds causing white or yellow flesh colour, respectively, as well as the absence of anthocyanins compounds causing red, blue, purple shades of flesh colour, irrespective of the presence of colour in patterns being partial or full.

The term "tuber taste" as used herein refers to the consumers appreciation by consuming a cooked potato tuber.

The term "tuber shelf life" and "tuber storage ability" as used herein are synonymous and refer to lack of changes in appearance of a tuber at storage.

The term "tuber dormancy period" as used herein refers to the time period between sowing and sprouting of a tuber, while sown at normal conditions.

The term "resistance to tuber dehydration" as used herein refers to a plant with shows less dehydration of the tuber than average at low humidity conditions (see shelf life).

The term "tuber starch content" as used herein refers to the starch weight over the total fresh weight of a tuber.

The term "tuber dry matter content" as used herein refers to the weight of the dry components of a potato divided over the total fresh weight.

The term "tuber cooking quality" as used herein refers to the continuous variation from firm to mealiness of a tuber after cooking.

The term "tuber frying quality" as used herein refers to the consumers appreciation of a tuber after frying.

The term "tuber chip making quality" as used herein refers to the consumers appreciation of a tuber after making chips.

The term "tuber uniformity" as used herein refers to the standard deviation of tuber traits, whereby a low standard variation refers to high uniformity and vice versa.

The term "cold sweetening resistance" as used herein refers to resistance of a tuber to produce glucose and/or fructose while stored at temperatures below 8 °C.

The term "tetraploid" as used herein refers to a plant wherein each vegetative cell contains four sets of chromosomes (4n). The terms "fertile" and "fertility" broadly refer to the capacity to reproduce, i.e. to conceive and to produce offspring, preferably fertile offspring. Thus, the term fertility refers to individuals that can be crossed to produce fertile offspring in that the flowers, when pollinated produce berries that contain seeds which when sown result in a plant that itself carries fertile flowers, etc. .

The term "fertile offspring" or "fertile seed" is defined herein as seed capable of growing into a flower-producing potato plant wherein flowers are male and female fertile. Thus, the term preferably refers to a plant or seed that when grown into a plant is capable of producing offspring as male and as female parent by virtue of the presence of fertile ovules and fertile pollen (i,e, both male and female flowers are fertile).

The term "fertile plant" is defined herein as a plant capable of producing fertile seed carrying berries. Preferably said berries each carry at least 5, more preferably at least 15-20, even more preferably at least 50 fertile seed, still more preferably between 50 and 500 seeds. Alternatively, a plant is said to be fertile when the number of seeds produced per berry is at least 10%, more preferably at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150% 200% or more of the number found in berries of the diploid breeding clone RH89-039-16. The term also, or alternatively, refers to plant lines having more than 25 %, preferably more than 90% fertile plants.

The term "self-compatible" refers to capacity to develop seeds in berries that are the result of self-pollination, self fertilization and producing fertile progeny.

The term "capable of being backcrossed/selfed/crossed and resulting in (self) fertile and self-compatible offspring" refers to the capability of producing one or more berries with seed as a result of backcrossing, selfing or crossing and wherein more than 60%, preferably more than 70%, 80%, 85%, 90% or more of said seed in said one or more berries is capable of developing into plants that are (self) fertile and self-compatible.

For other terms as used herein, reference is made to Allard, R. W. Principles of Plant Breeding, 2nd Edition, Wiley New York, 1999, and specifically to the Glossary therein.

Description of the preferred embodiments

The present inventors discovered that elite breeding lines for potato breeding can be successfully produced. Crossing of two elite breeding lines provides F1 hybrid seeds, such F1 hybrid seeds, when grown into plants, result in plants of superior agronomic performance. More importantly, all seeds are genetically essentially identical, meaning that, except for the occasional crossover or mutation event, all seeds are uniform.

The present inventors have discovered a method that allows the provision of elite potato breeding lines, which, when crossed mutually, provide uniform F1 hybrid potato seeds which, when grown into plants, result in plants having superior agronomic performance. The elite potato breeding lines may have complementary traits and, as a result of their homozygosity, the F1 plants grown from the F1 hybrid seeds are uniform, but when these F1 plants are subsequently selfed or cross-pollinated the resulting F2 plants will segregate for the various traits.

Thus, in contrast to the currently available true potato seeds (TPS) generated in the conventional ways of the prior art and harvested from berries in which every individual seed is genetically different from every other seed, the seeds obtained from crossing of the

potato lines and elite potato breeding lines in accordance with aspects of the present invention are genetically essentially identical.

The TPS varieties of the prior art are derived from crosses between selected but heterozygous parents and hence variability is expected in plants and tubers. Hybrid potato breeding as proposed herein differs from the conventional production of TPS in that the present TPS are the result of the crossing of two pure breeding lines, resulting in uniform cultivars, whereas TPS varieties of the prior art are the result of open pollination or crossing between two genetically non-homozygous and often tetraploid breeding lines resulting in non-uniform cultivars.

Not only are the presently provided plants novel in that they combine a number of properties, heretofore never attained together in a single potato line, they also allow for novel and very advantageous breeding methods. Hence, the plants of the invention are an intermediate in the production of novel potato cultivars, which cultivars can be produced by methods as disclosed herein. The advantageous breeding methods allow for the elimination of many of the drawbacks of conventional potato breeding as indicated in hereinabove including the elimination of the time-consuming selection of clones to arrive at a new potato variety and the possibility to provide uniform hybrid potato seed. In addition, the novel plants and methods also allow for the elimination of drawbacks related to clonal propagation and production, including the possibility to eliminate high transportation costs and the clonal persistence of pests and diseases.

Novel potato plants

Parental breeding lines

The present invention provides potato plants that are essentially homozygous, diploid, vigorous, fertile and self-compatible.

Preferably the plants of the invention have fixed within their genome nucleic acid sequences encoding for genes that confer to the plant an agronomically desirable trait. Such nucleic acid sequences may be introgressions or transgenes derived from potato germplasm with agronomically desirable traits, preferably from diploid germplasm.

- Essentially homozygous

In breeding, plants are propagated by sexual reproduction, usually by crossing of selected parents with desirable traits to produce improved progeny. Progeny inherit genes for both desirable and undesirable traits from both parents. Repeated selection of meritorious individuals from each generation that will serve as parents of the next will

conserve desired characteristics and eliminate undesirable ones. The ultimate parent in plant breeding is a purebred, a highly homozygous line that, when used in crossings with other purebreds results in offspring that is identical, both phenotypically and genotypically, within a single generation and between consecutive generations obtained from other crossings of the same parents. Purebreds, or pure inbreds, can be obtained by everal generations of selfing or inbreeding (crossing of close relatives, such as brother to sister plants or offspring to parent (backcrossing)) each successive generation showing an increased degrees of genetic uniformity, or homozygosity. The use of homozygous inbreds as breeding parents results in offspring that is identical, both phenotypically and genotypically, within a single generation and between consecutive generations obtained from other crossings of those parents. Hence, the pure inbreds can be used repeatedly to produce the same offspring plants.

In potato, selfing and inbreeding results in severe inbreeding depression, or the expression of deleterious recessive genes, which expression is revealed by loss of vigour and loss of fertility. Hence, it has hitherto been impossible to produce homozygous pure inbreds in potato since until the present invention, high levels of homozygosity in potato with sufficient vigour could not be attained. The present invention now, for the first time, provides plants having a level of homozygosity that is above 25%, and preferably above 75 % and that is combined with fertility, self compatibility and plant vigour.

- *Diploid*

The plants of the present invention are diploid plants, in contrast to the commercial potato varieties, which are tetraploid. It should be noted that in plants of the present invention higher ploidy levels are expressly envisaged, since the diploid stage is used primarily during the development of the breeding lines. Once the purebreds have been established, the lines may be made tetraploid. Hence, plants of the invention may also be tetraploid. Tetraploid plants can be produced from tetraploid cells or tissue cultures, which in turn may be produced by duplicating the genome of diploid cells or tissue cultures by methods known per se, for instance by using oryzalin as described by Barandalla *et al.* (Barandalla et al. Potato Research (2006) 49:143-154). Tetraploid plants may have the advantage that they provide higher yields.

- *Vigorous*

Although commercial tetraploid potato varieties are fertile and self-compatible and vigorous in that they grow well, do not produce deformed plants, or fruits, but manifest as healthy plants with a high tuber yields, their selfed progeny shows serious inbreeding

depression, that is, result in faint growing and low yielding plants with deformed tubers. Prior art diploid and essentially homozygous lines, such as those reported by Phumichai et al. as referred to herein, also grow only very poorly, and manifest as very weak and small plants, that have poor seed set. In fact, diploid potato plants resulting from inbreeding, and attaining homozygosity of at least 25 % or 50% on the alleles are invariably weak growers.

The plants of the present invention are not weak and frail, and although they need not necessarily be as vigorous as present day commercial tetraploid varieties, they do not show inbreeding depression. Hence, within the context of the present invention, they are vigorous. Tuber formation exemplifies the vigour of the plants of the present invention. Although all commercial (tetraploid and seed-tuber derived) potato varieties produce large tubers, or at least tubers of sufficient size, prior art diploid lines and essentially homozygous plants produce only small tubers, which are, for instance, of no use in the production of potato chips or French fries. Prior art diploid lines and essentially homozygous plants produce also very few tubers such that the yield of tubers per plant is at least ten times lower than that of heterozygous diploids plants and commercial tetraploid potato varieties. In contrast, the plants of the present invention exhibit beneficial tuber formation characteristics, including tubers of beneficial size profile, and a tuber yield that may be at least 50% compared to the diploid heterozygous plants used as donors in the examples as described herein (D1, D2, and/or D3).

As was found herein, the tuber yield of the diploid F1's was on average 443 grams per plant, with values as high as 600 and 634 grams.

Backcrossing with the F1: "IVP08-06" yielded on average 155 and 209 grams, while backcrossing with the F1: "IVP08-07" yielded on average 209 and 259 grams.

The "pseudo F2's" yielded on average 172 and 332 grams per plant, and occasionally produced higher than the diploid F1's.

The F2's yielded on average 69 and 252 grams per plant. Several plants overlap with the yield values of the diploid F1s.

- *fertile*

The plants of the present invention – although essentially homozygous – are also fertile. That means that they can be used in breeding programs because their offspring will also be fertile.

- self compatible

The plants of the present invention are self compatible. This feature is introduced in order to allow the selfing of plants and the production of homozygous lines.

The preferred basis of the self compatibility is derived from the dominant S locus inhibitor gene (*Sli*) (Hosaka and Hanneman, 1998, Euphytica, 99: 191–197).

The essentially homozygous potato plants of the present invention have as an advantage that they can be crossed with other essentially homozygous potato plants whereby the hybrid offspring of said cross is essentially genetically uniform and will not segregate.

The present invention, in a preferred embodiment, provides essentially homozygous, diploid, vigorous, fertile, and self-compatible potato plants having agronomically desirable traits.

The novel potato plants of the invention are capable of:

- being backcrossed to an essentially homozygous recurrent parent and resulting in (self) fertile and self-compatible offspring;
- being selfed (self-fertilization of 1 offspring individual) and resulting in (self) fertile and self-compatible offspring;
- being mutually crossed (fertilization between 2 offspring individuals) and resulting in (self) fertile and self-compatible offspring.

Plants according to the present invention include the line with breeders reference AGVD1, representative seeds of said line having been deposited on 23 October 2009 with the NCIMB, Aberdeen, Scotland under accession number NCIMB 41663 and breeders reference AGVD1, which plant represents an F2 obtained by crossing a plant of parent IVP07-1001/4 (see Example 1), which is an essentially homozygous, diploid, fertile and self-compatible but non-vigorous potato line with a plant of parent D1, which is an elite diploid breeding clone that is essentially non-homozygous, and is not self-compatible, but which has the desirable agronomic trait of yellow flesh color and exhibits good cooking quality in that the flesh is not greying after cooking, and selfing the resulting F1 offspring plants. It is estimated that AGVD1 is about 50% homozygous, meaning that about 50% of the loci for which the parents differ are homozygous.

Plants according to the present invention also include the line with breeders reference AGVD2, representative seeds of said line having been deposited on 23 October 2009 with the NCIMB, Aberdeen, Scotland under accession number NCIMB 41664 and under breeders reference AGVD2, which line represents an (F1 x F1) line (or pseudo F2) obtained by crossing a plant of parent IVP07-1001/4 as indicated above with a plant of parent D1 as indicated above, and mutually crossing individual F1 offspring plants, that share the IVP07-1001/4 parent but have different individuals of the D1 parent. It is

estimated that AGVD2 is about 25% homozygous for the loci for which the parents differ.

Plants according to the present invention also include the line with breeders reference AGVD3, representative seeds of said line having been deposited on 23 October 2009 with the NCIMB, Aberdeen, Scotland under accession number NCIMB 41665 and under breeders reference AGVD3, which line represents a BC1 population obtained by crossing a plant of parent IVP07-1001/4 as indicated above with a plant of parent D1 as indicated above, and backcrossing the resulting F1 offspring plants with the IVP07-1001/4 parent. It is estimated that AGVD3 is about 75% homozygous for the loci for which the parents differ.

Plants according to the present invention also include the line with breeders reference AGVD17, representative seeds of said line having been deposited on 5 October 2010 with the NCIMB, Aberdeen, Scotland under accession number NCIMB 41765 and under breeders reference AGVD17, which line represents an F3 population obtained by crossing a plant of parent IVP07-1001/4 as indicated above with a plant of parent D2 as indicated above, and backcrossing the resulting F1 offspring plants with the IVP07-1001/4 parent, followed by two steps of selfing. It is estimated that AGVD17 is > 80% homozygous for the loci for which the parents differ.

A plant of the present invention can very suitably be used as a recurrent parent wherein additional agronomically desirable traits are introgressed by crossing, or introduced by other means such as by transgenic means. Once a number of agronomically desirable traits have been stacked into an essentially homozygous, vigorous, diploid, fertile and self-compatible plant according to the invention, that plant can be used as an elite breeding line for the production of commercial F_1 hybrid potato seed. Likewise, a second elite breeding line can be provided that can serve as the parent of opposite sex so that both plants can be crossed to produce the commercial F_1 seed of a novel potato variety.

The breeding of uniform hybrid seed depends on a number of technical conditions. Prerequisites to achieve uniform hybrid seed are that i) an initial introgression crossing can be made between an essentially homozygous, diploid, fertile and self-compatible plant and a plant that acts as donor for an agronomically desirable trait, that ii) a backcross can be produced between the offspring of that initial crossing and the essentially homozygous, diploid, fertile and self-compatible plant, and that iii) the offspring thereof can be selfed. Potato lines AGVD1, AGVD2 and AGVD3 exhibit these characteristics and individual plants of these lines can be used in additional introgression crossings, backcrossings and selfings to arrive at the elite breeding lines suitable for use as

parents for commercial F1 hybrid seed that can be grown into hybrid potato plant of a new potato variety.

A highly preferred plant according to the present invention is a plant of line AGVD2 or an offspring plant thereof having all of the physiological and morphological characteristics of the plant of line AGVD2.

Another line deposited as example of embodiments of the present invention is AGVD17 (NCIMB 41765, deposited 05 October 2010). The advantage of AGVD17 is that it is a highly homozygous line (> 80% homozygous) combining vigour and fertility with a tuber yield of on average 300 grams (fresh weight) per plant. Individual plants of related populations even showed tuber yields of 500 grams per plant. These data were generated from field grown plants that were raised from seedlings. Potato plants raised from clones generally yield tuber weights that are 50 to 100% higher than plants raised from seedlings. Hence, the tuber yield expressed in grams of fresh weight from plants as claimed herein – when clonally propagated and raised from tubers and grown in clay soil under normal North Western European summer conditions – is expected to reach levels of at least about 1000 grams fresh weight per plant.

Thus, line AGVD17 demonstrates in particular the possibility to intercross individual elite lines of the present invention (i.e. that are self-compatible, fertile, and essentially homozygous) to arrive at the hybrid seed that is the aim of the present invention. Hence, another highly preferred plant according to the present invention is a plant of line AGVD17 or an offspring plant thereof having all of the physiological and morphological characteristics of the plant of line AGVD17.

Hybrids

The hybrid plants of the invention are produced by a method of the invention as exemplified in Figure 1. The hybrid plants of the invention are produced by crossing two essentially homozygous elite potato parental breeding lines comprising among them a number of agronomically desirable traits selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, cold sweetening resistance and resistance to tuber dehydration. In addition to these agronomically desirable traits, the parental breeding lines may exhibit such characteristics as desired in specific use markets such as desirable tuber skin color, desirable tuber shape, desirable tuber flesh color, desirable tuber taste, desirable tuber starch content, desirable tuber dry matter content, desirable tuber cooking quality, or

desirable tuber frying quality. In addition to such market-specific characteristics, the parental breeding lines, when crossed, preferably result in hybrids that exhibit such beneficial traits as beneficial growth rate, beneficial tuber yield, beneficial tuber size, beneficial tuber storage ability, beneficial tuber dormancy period, beneficial tuber shelf life, beneficial tuber chip making quality, and beneficial tuber uniformity.

The present invention now provides a set of potato plants or a set of potato plant lines, which plants or plant lines are: diploid (2n); essentially homozygous (preferably more than 60% on all alleles); self compatible; fertile; and vigorous. Hitherto it has proven impossible to provide the combination of all these features in a single potato plant (line). The set of potato plant of the invention can be used to produce novel varieties or cultivars.

The invention particularly relates to a set of at least 2 diploid (2n), essentially homozygous (preferably more than 60% on all alleles), self compatible, fertile, and vigorous plants that are crossable to produce hybrid varieties according to the invention.

Novel method of potato seed breeding

The provision of the novel potato plants now provides for the first time the attainment of the method of the invention.

The present inventors have generated potato plants that are essentially homozygous, diploid, vigorous, fertile and self-compatible and having fixed within their genome nucleic acid sequences encoding genes that confer to the plant an agronomically desirable trait derived from suitable diploid donor lines. The said nucleic acid sequences are the result of introgressions from potato germplasm having agronomically desirable traits. Conventionally, at least in most species other than potato, homozygous plants can in principle be obtained in as little as two generations by doubling of monohaploids generated from unfertilised generative cells such as egg or pollen cells by methods known as doubled haploid (DH) technology. This is not possible in potato, as the resulting plants have hitherto shown to be infertile, due to - what is considered – expression of recessive deleterious genes. The DH method was extensively tested by the present inventors, but was found unsuccessful.

The present inventors used the strategy of repeated selfing, backcrossing and crossing of potato germplasm of a single self-compatible potato line and one or more self-incompatible potato lines harbouring agronomic traits. The homozygous plants of the present invention were obtained by continuous selfing of diploid potato plants and selection for fertile offspring (besides the inherent selection for self-compatibility). This is an entirely new approach, as in the prior art methods, plants are obtained by selection for

agronomical traits, such as tuber formation or general growth characteristics of the offspring plants, not by selection for fertile offspring from selfings. Moreover, and unlike the prior art methods the procedures are performed using diploid plant lines, instead to tetraploid plants. Unexpectedly, this radically new approach proved successful for obtaining increasingly homozygous, diploid offspring plants, that were self-compatibel and fertile. In addition, when (out)crossed with plants of diploid lines harbouring beneficial traits, such as resistance or white tuber color, these traits could be introduced into the essentially homozygous germplasm without loss of self-compatibility and fertility.

In essence the method for obtaining the plants is as follows:

Starting point is a diploid self-compatible potato line (examples of suitable lines have been deposited under depositors reference AGVD 1, AGDV 2, AGDV 3 and AGVD17). In general a line being self-compatible due to the presence of the *Sli* gene may be used as starting point. Plants of this diploid self-compatible line are selfed and seeds obtained from seed-carrying berries are sown and grown into offspring plants. Offspring plants carrying flowers are again selfed and seeds obtained from seed-carrying berries are again sown and grown into offspring plants. No, or at least little, attention is paid to the agronomical appearance of the plants at this stage. They may look withered and frail, with only a few flowers. Nonetheless, the selfing is repeated at least three times, but preferably 4 or 5 or even 6-8 times. In this manner a line of essentially homozygous, fertile, self-compatible, and diploid potato plants is obtained, essentially by selfing and selecting for plants that produce fertile offspring.

Next, an agronomically desirable trait is added to the essentially homozygous, fertile, self-compatible, diploid potato genotype by crossing a plant of that line with a plant of an unrelated essentially heterozygous diploid potato line, preferably a line containing an agronomically desirable trait. This essentially heterozygous line (which term merely reflects that the line is not an essentially homozygous line as used herein) functions as a donor for an agronomically desirable trait, in this case vigour.

The resulting hybrid is selfed and/or backcrossed to the self-compatible line while selecting for higher vigour. In this way, the vigour of the essentially homozygous, fertile, self-compatible, diploid potato plant line is enhanced and at the same time the line is agronomically improved. These steps will thus result in a vigorous fertile homozygous self-compatible diploid potato: The vigour may further be enhanced by crossing with other essentially heterozygous diploid potato lines, which additional lines serve as donors for additional agronomically desirable traits, followed by selfing and/or backcrossing to to the self-compatible line while selecting for these additional traits, which additional steps will result in the provision of diploid potato breeding lines.

In order to produce elite diploid potato breeding lines, the breeding value of the breeding lines is further enhanced by intercrossing, selfing and backcrossing while continuous selecting for the agronomically desirable traits provided to the breeding line, and by establishing lines that exhibit good combining abilities with other lines. Such combining abilities are evaluated by the use of experimental crosses.

Finally, hybrid potato cultivars can be produced by crossing individual elite diploid potato breeding lines that exhibit good combining abilities. The seeds resulting from this cross of homozygous parents are hybrid true potato seeds that are uniform.

Using the methods of the present invention, generally, around 7 to 8 selfing cycles will result in essentially homozygous plants, but the skilled artisan will understand that more generations of selfing may also be used. Also fewer generations, such as six generations may be used to provide essentially homozygous plants of the present invention. The number of backcross generation can even be reduced to three to reach more than 95% homozygosity when applying molecular genetic markers.

Essentially, the novel method of producing hybrid potato cultivars provides for the possibility to produce a new potato variety within about 5 years. A detailed process for the production of hybrid potatoes starting from AGVD17 (F3 line) is provided in Example 5. This line is >80% homozygous such that very few selfings are needed to obtain a plant that is about 95%-100% homozygous. In addition AGVD17 is already vigorous as defined herein.

The present invention now provides a method for producing a genetically homogenous or uniform true potato seed (TPS). This method is *inter alia* useful in a method of preventing transmittal of virus infections between potato generations, but also for overcoming many of the other problems associated with classical potato breeding, "seed" potato production and transport and cultivation. Moreover, the present parental material for production of hybrid seed can be maintained in the form of seed, and not in the form of vegetative material as is required by traditional TPS production.

The present invention provides in one aspect a method for producing a hybrid potato cultivar comprising the use of the deposited plant material provided herein as a starting material.

Alternatively, other essentially homozygous, diploid, fertile, vigorous and self-compatible plant lines different from the deposits referred to herein may be developed and the production of a hybrid potato cultivar can commence from that early stage level by using the teaching provided herein since the present inventors have demonstrated that this

route, although laborious, is successful. Such an alternative breeding material is also vigorous, essentially homozygous, diploid, fertile and self-compatible.

As a first step, such an early stage development may comprise the provision of a plant of a first cultivated potato species (*Solanum tuberosum*) or a related crossable species (generally a tuber bearing *Solanum* species).

Next, said plant is developed into a line of self-compatible diploid potato plants, preferably a *Solanum tuberosum*. Diploid potato is usually self incompatible. Self-compatibility may suitably be introduced from related species (see for instance Hermsen, J.G.Th. 1978, Euphytica 27, 1-11).

The resulting line of self-compatible diploid potato plants is then developed into a homozygous fertile self-compatible diploid potato line. Homozygous diploids can be obtained by doubling haploids (Uijtewaal et al., 1987, Theor. Appl. Genet. 73, 751-78) by anther culture (Jacobsen and Sopory, 1977, Theor. Appl. Genet. 52, 119-123), by ovule culture or by repeated selfings (Phumichai & Hosaka, 2006, Euphytica 149, 251-258). The latter method was used for producing the homozygous fertile self-compatible diploid potato line that formed the basis of the vigorous plants as described herein.

Next, the homozygous fertile self-compatible diploid potato line is developed into a vigorous fertile homozygous self-compatible diploid potato line. The vigour may be enhanced by crossing with other diploid potato plants, selfing and backcrossing while selecting for higher vigour. It must be noted that high populations are needed and that even for some unrelated essentially heterozygous diploid potato lines that are used as donor of the agronomically desirable trait, very few self compatible plants may be found in a population of 1000 plants. Generally, such attempts are terminated, and only when about 10-50% of the offspring plants is self-compatible (such as in the case of D2 in Example 2 below) the donor line exhibits such good general combining ability that a successful introduction of the agronomical trait into the self-compatible lineage is foreseen.

Next, the vigorous, fertile, homozygous, self-compatible, diploid potato line is developed into a diploid potato breeding line. Most preferably, at least two separate vigorous fertile homozygous self-compatible diploid potato lines are produced and at least two distinct diploid potato breeding lines are developed. Such breeding lines comprise a high number of agronomically desirable traits. Agronomically desirable traits are introduced by crossing with other diploid potato plants, selfing and backcrossing while selecting for these traits. This breeding program may comprise testing for disease, such as whether the shoots or tubers are free of disease, or whether they are resistant for disease.

Next, the diploid potato breeding lines are developed into elite diploid potato breeding lines. This is accomplished by enhancing the breeding value by intercrossing,

selfing and backcrossing while continuously selecting for agronomically desirable traits. At this stage experimental crosses are performed to evaluate general combining abilities. The diploid elite lines can now also be developed into tetraploid (4x) elite lines by methods known per sé, such as by chemical treatment with e.g. colchicine, or by "spontaneous" doubling of the genome using tissue culture.

Finally, potato hybrid cultivars are produced from these elite diploid or tetreploid potato breeding lines by crossing of good combining breeding lines. The seeds obtained are hybrid true potato seeds that are uniform.

The method of the invention for producing a potato hybrid cultivar comprises the steps of producing a diploid potato breeding line. Said method comprises the steps of:

(a) providing a first potato plant, which first potato plant is a plant of a diploid, self-compatible and essentially homozygous potato line, preferably a plant of a vigorous fertile homozygous self-compatible diploid potato line, more preferably a diploid potato breeding line;

(b) providing a second potato plant of a potato line that may have any level of ploidy, preferably diploid, and/or any level of homozygosity wherein said second potato plant serves as a donor for an agronomically desirable trait which trait is to be introgressed into said essentially homozygous line of said first potato plant or at least in an offspring plant resulting from a crossing between said first and second plant, wherein said trait is selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, rooting ability, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, growth rate, tuber development defects (e.g. misshapen or damaged tubers), tuber yield, tuber size, tuber skin color, eye depth, tuber shape, tuber flesh color, tuber taste, tuber storage ability, tuber dormancy period, tuber shelf life, resistance to tuber dehydration, tuber starch content, tuber dry matter content, tuber cooking quality, tuber frying quality, tuber chip making quality, tuber uniformity and cold sweetening resistance.

Following the provision of these two plants, the method then comprises the step of:

(c) cross-pollinating said first and second potato plant such that one of said parent plants produces seed-carrying fruits (berries) and collecting said seeds from said fruits to provide a hybrid offspring seed.

Seeds are then grown to plants and the plants that comprise the agronomically desirable trait from the donor plant are selected, either directly or following additional

selfing and backcrossing steps, as is well known in the art of plant breeding. In these methods markers may be used to assist in the breeding process.

The hybrid offspring seed is preferably grown into a plant that is subsequently backcrossed a plant to the recurrent parent line

The following steps are not essential to either of the above methods. However, these additional steps may either provide for the production of consumption tubers by growing the seeds of the invention and/or may provide for the production of new homozygous, self-compatible, diploid elite breeding lines that can serve as parents for the inclusion of yet further traits into an offspring generation. The plants of the invention are fertile and vigorous. These additional benefits are attained by the following further steps of:

(d) growing said offspring seed into a hybrid offspring potato plant, and

(e1) harvesting tubers from said hybrid offspring potato plant to provide tubers carrying said trait, or

(e2) selfing said hybrid offspring potato plant or backcrossing said hybrid offspring potato plant to a potato plant of said line of said first potato plant to provide further offspring plants carrying said trait and wherein said of offspring plants are increasingly homozygous following each backcrossing or selfing step.

Preferably said second potato plant used in the method described above is a hybrid offspring potato plant as defined in step (d) above and obtained by the method as described above. More preferably, said second potato plant used in the method described above is a hybrid offspring potato plant as defined in step (e2) above and obtained by the method as described above.

Preferably, said step (e2) comprises from 2 to 7 backcrosses using a plant of line AGVD1, AGVD2, AGVD3 or AGVD17 as the recurrent parent and testing offspring plants for the presence of the trait (e.g. in the form of the homozygous or heterozygous presence of alleles responsible for the trait), followed by one or two (or more) selfings, to provide an essentially homozygous plant that is genetically equal to a plant of population AGVD1, AGVD2, AGVD3 or AGVD17, but in which alleles responsible for the trait are heterozygously, preferably homozygously present. This plant has at least one additional allele not available in population AGVD1, AGVD2, AGVD3 or AGVD17.

The above method now provides a breeding process for the production of homozygous breeding lines or elite breeding lines based on the background of population AGVD1, AGVD2, AGVD3 or AGVD17, to which background successively additional alleles are added, thereby providing elite breeding lines wherein desirable traits are stacked.

The method of the present invention now for the first time provides for a method for preventing transmittal of virus infections between potato generations while at the same time also solving many of the other problems of the prior art related to breeding of new potato varieties and commercial production of potatoes for consumption. This has become possible through the provision of true potato seed, i.e. botanical seed, which seed can be grown to a potato plant that produces tubers for consumption, and which seeds are from a cross in which at least one of the parents is a plant of a diploid, self-compatible and essentially homozygous potato line, preferably said seeds are from a cross wherein both of the parents are plants from a diploid, self-compatible and essentially homozygous breeding line.

An important advantage of this new method is that, in this way, the transmittal of infection with a virus that is not spread through the gametes and seeds can effectively be prevented.

The present inventors now provide a method for further breeding for homozygous lines that are easy to handle, and which exhibit improved fertility and plant vigour when compared to prior art inbreds, while maintaining the self-compatibility and high level of homozygosity. It will be appreciated that – just as in tomato - a 100% homozygosity is not always needed to provide for an effective breeding program. This is not a problem as long the self-compatibility is maintained.

The genetic transfer of agronomically desirable or beneficial traits into a homozygous potato line has now for the first time been successfully shown.

Based on the means and methods provided herein, the skilled person will be able to successfully produce homozygous potato breeding lines based on his own germplasm. In addition, the skilled person will appreciate that means and methods are provided by which he is able to, for instance, stack additional traits into the presently provided self-compatible and essentially homozygous lines in order to establish a population of lines that are enriched for agronomically desirable traits and that can serve as germplasm for commercial breeding in their own right.

EXAMPLES

Example 1. General description of the breeding procedure and pedigrees.

Many attempts were made to generate homozygous, fertile, self compatible diploid genotypes of potato without success. These attempts included the formation of doubled haploids, and the subsequent screening for fertile flowers. Several methods were employed like anther culture to regenerate haploid and eventually double haploid homozygous plants, repeated selfings to gradually increase the level of homozygosity in existing diploid germplasm and prickle pollination by using S. phureja as pollen donor to induce embryo formation from unfertilized egg cells. Especially the latter method was done at a large scale, by analyzing more than two million offspring but without the desired success. None of these tests resulted in vigourous self compatible plants.

An essentially homozygous potato line was obtained by multiple generations of selfing of plants of a line derived from public germplasm which plants have the self-compatibility controlling *Sli* gene (Phumichai et al. Euphytica (2006) 148: 227–234). These plants, designated "H" or IVP07-1001/4 herein, grew poorly, had very few flowers and each berry contained only few seeds. Moreover, the plants were very small (max 60 cm). The plants produced almost no tubers and those that formed were very small (average tuber weight about 5 grams/tuber). In all, the plants were of no value to commercial potato breeding, as they exhibited a plant weight and tuber yield that was less than 20% of that of diploid breeding clone RH89-039-16. The plants merely contained homozygous alleles on many of the loci in their genome, were self-compatible and diploid.

In the summer of 2008 plants of this IVP07-1001/4 line were crossed with different diploid donor lines (D1, D2 and D3, See for complete pedigree figure 2). D1 is early, has long sized oval tubers, light yellow flesh, and good cooking fries making quality and good cooking quality and was used as male parent (Table 6). The resulting progeny is referred to as F1(D1) in this example. Other F1 offspring plants were obtained in the similar way but using donor D2 as a female parent. D2 is an elite diploid breeding clone that is essentially non-homozygous, and is not self-compatible, but which has the desirable agronomic trait of early cooking, oval tubers, yellow flesh and good cooking chips making quality). This resulted in lines designated as F1(D2).

The above F1's were (D1) was selfed to produce an F2(D1). This line was deposited under breeders reference AGVD1. It is estimated that AGVD1 is about 50% homozygous, meaning that about 50% of the loci for which the parents differ are homozygous.

F1(D1) was crossed with F1(D2) to produce a pseudo F2. This line (F2(D1/2)) was deposited under breeders reference AGVD2. It is estimated that AGVD2 contains about 25% homozygous alleles originating from IVP07-1001/4.

The F1's were(D1) was also backcrossed with IVP07-1001/4 to produce a BC1's line. This line (BC1(D1) and BC1(D2)) was deposited under breeders reference AGVD3. It is estimated that AGVD3 is about 75% homozygous for the loci for which the parents differ.

Other F1 offspring plants were obtained in a similar way by using donor D3 as female parent. D3 is a diploid breeding clone with yellow flesh and with the resistance genes R3, H1, Gpa2, RXadg. This resulted in a line designated F1(D3).

Similarly as described above, F1(D3) was selfed to generate an F2(D3) and backcrossed with the Sli gene donor to generate BC1(D3) offspring.

F1(D1) was crossed with F1(D2) to produce a pseudo F2. This line (F2(D1/2)) was deposited under breeders reference AGVD2. It is estimated that AGVD2 is about 25% homozygous for the loci for which the parents differ.

F1(D1) was also backcrossed with IVP07-1001/4 to produce a BC1 line. This line (BC1(D1)) was deposited under breeders reference AGVD3. It is estimated that AGVD3 is about 75% homozygous for the loci for which the parents differ.

The BC1's were selfed in a winter nursery resulting in BC1S1 populations. Next summer (2010) the F2's, the pseudo F2's and the BC1S1's were sown in seed beds in a nursery and transplanted in small peat pots. In June 2010 the seedlings were transplanted to the field at a clay and at a sandy soil in The Netherlands. In total more than 15000 plants were grown from seedlings and selected for self compatibility and for good tuber quality and yield. Most F2 and BC1S1 populations showed a very low frequency of self-compatibility. Rare plants with reasonable good berry- and seed set were selected for progeny testing without further agronomic evaluations. Only two F2 populations, originating from the D2 x H cross, showed about 25% self compatibility. For each of the most self-compatible populations maximally fifteen plants with the best tuber quality and quantity were selected for progeny testing.

Plants of the pseudo F2's showed much more vigour than the other populations, probably as these plants do not harbour any locus with homozygous alleles of the donor parents. One of these populations showed more than 50% self-compatibility. Similarly as described above, also maximally fifteen plants of the most self-compatible populations were selected for progeny testing.

In addition, crosses were made with self-compatible plants from the field as pollen donor on two to twelve plants of the donors D1-D19 (Example 3). After evaluation

of the tuber quality and quantity only crosses with selected plants or their full sibs, in case of lack of sufficient numbers of crosses, were selected for progeny testing.

In this way, in each generation, plants are selected for self-compatibility and good agronomic performance. Seeds of the selfed berries of these selected plants are collected for further testing in order to generate homozygous lines. In addition, crosses of the selected plants with other diploid donors or other breeding lines are made to enhance the genetic variation. These F1 seeds are heterozygous and several rounds of inbreeding are needed to generate homozygous offspring with the desired traits.

In this way two generations of selections, selfings and crosses can be done per year. This process is even further enhanced by exploiting marker aided breeding by using diagnostic markers for specific traits and random markers to select for the recurrent parent genome. When elite parents have been obtained, derivatives of these lines can be made by two to three generations of backcrossing and two generations of selfing.

Example 2 Breeding self compatible diploid potato.

1. Introduction

A common characteristic of diploid potato is self-incompatibility (SI); upon self-fertilisation no vital progeny is generated. The S-locus inhibitor gene (*Sli*) originating from *Solanum chacoense* has been described (*Euphytica* 99,191-197, 1998) to inhibit this self-incompatibility and rendering diploid potato self-compatible (SC). Upon repeated selfings, close to homozygous self-compatible diploid potato clones have been generated (Genome 48, 977-984, 2005). The agricultural quality of these clones was very poor indeed reflecting a poor germination, plant growth, tuber quality and tuber yield.

2. Experiments

2.1 Materials

In order to develop self-compatible potato clones with much higher level of agricultural traits we crossed the *Sli* gene donor with various potato clones, designated: IVP97-079-9, IVPAA-096-18 and SH83-92-488. These clones are available at Dr. R.C.B. Hutten, Laboratory of Plant Breeding, Wageningen University, The Netherlands. As used herein, these clones are designated as clones D1, D2 and D3 resp. The progenies (F1) were selfed and backcrossed to the *Sli* gene donor generating F2 and BC1 populations, respectively. The BC1 was selfed to generate BC1S1 populations. All these crosses and

self fertilisations were performed without any other selection than self compatibility of the plants.

2.2 Evaluation of progenies in a wintertrial

In a pilot experiment several random BC1 and F2 progenies were grown in a heated greenhouse with 16 hours artificial light in while outside temperatures were below zero for almost three months. We evaluated the self-compatible % as the number of plants with berries after hand pollination and the yield as the weight of tubers per plant (Table 2).

Results

The *Sli* donor is self-compatible (SC) and most of the F1 and BC1 plants were also self-compatible, while a minority of the F2 plants was self-compatible. The *Sli* donor showed an extremely low tuber yield, while the BC1 and one F2 was about 50% of the F1 tuber yield. The tuber yield of some BC1 plants was close to that of the F1 plants and some (unselected) F2 plants were better than the weakest F1 plants.

Table 2. Some characteristics of winter grown plants of a diploid potato breeding program to introduce the *Sli* gene.

	# plants	compatibility	yield (gram per SC plant)	
		frequency (%)	average	variation
Sli donor	2	100	22*	0 - 43
F1 (HxD3)	9	89	443**	258 - 634
BC1 (Hx(HxD1)	49	81	206**	0 - 524
BC1 (Hx(HxD2)	64	72	237**	28 - 543
F2 selfed (HxD1)	23	22	252**	131 - 376
F2 selfed (HxD2)	20	25	69**	0 - 201

* from tuber

** from seedling

2.3 Evaluations of progenies in the field

In the summer of 2010 progeny plants of F2 and BC1S1 populations were tested in the field to select self-compatible progeny plants with good agricultural performance (Table 3).

Table 3. Breeding scheme to introduce self-compatibility (SC) into diploid potato. In bold the most promising materials are indicated.

P	F1 (HxD)	F2: selfed (HxD)				BC1S1: selfed (Hx(HxD))			
parents	plants (DxH)	Populations tested	plants in the field	self compatible plants	plants selected*	populations tested	plants in the field	self compatible plants	plants selected
	#	#	#	#	#	#	#	#	#
H = *Sli* donor									
D1	33	15	2460	± 50	7	38	2521	< 10	1
D2	45	29	***5598***	***> 500***	***52***	24	1380	< 10	1
D3	8	8	1602	± 10	7				

* sufficient seedset

Results

We tested 52 F2 populations comprising 30 to 840 plants and 62 BC1S1 populations comprising 1 to 390 plants, in total 9660 F2 plants and 3901 BC1S1 plants resp. Most of the populations showed a weak plant growth and a low frequency of self-compatibility. Only two F2 populations derived from the D2 parent showed about 25% self compatible plants. The best plants of these populations were selected on the basis of tuber yield and tuber quality.

3. Conclusion

Only two out of the 52 F2 populations and none of the BC1S1 populations showed a reasonable level of self-compatibility. However, unexpectedly, the desired plants could be obtained, starting from material that would otherwise have been neglected by breeders. The main differences of the plants of the invention and those of the prior art are given in Table 4 below.

Table 4. Characteristics of the parental potato lines of the present invention proposed for the production of hybrid (true) potato seed.

	Vigour	**Ploidy**	**Tuber yield**	**Fertility**	**Self-compatibility.**
Sli-gene donor (Phumichai et al. Euphytica (2006) 148: 227–234).	low	2n	low	low/ intermediate	high
Diploid trait Donor (e.g. D1)	intermediate	2n	intermediate	intermediate	low
Commercial variety	high	4n	high	high	low
Lines of present invention	Intermediate /high	2n	Intermediate /high	low/ intermediate	high

Example 3 Development of a molecular marker platform

Introduction

Numerous marker systems are available to support genetic research. For the present invention a molecular marker platform in potato was developed and used to evaluate the level of homozygosity versus heterozygosity in diploid potato.

Selection of most informative sequences for SNP assays

In order to analyse SNPs in potato, the Illumina standard GoldenGate protocol (Shen et al. 2005. Mutation Research 573:70–82) has been used previously (Anithakumari et al. 2010. Mol. Breeding, 26:65-75). This platform comprises 384 SNPs and was later expanded with another 768 SNPs. These 384 and 768 SNPs have been developed using EST sequences, which represent the mRNA without intron sequences. Since the present inventors have access to the first draft sequence of the potato genome (www.potatogenome.net) it was possible to compare the mRNA with the genomic DNA sequence including the intervening sequences. This comparison with the potato genome sequence was performed with the Basic Local Alignment Search Tool (BLAST; Altschul et al. 1990. J. Mol. Biol. 215: 403-410). This resulted in 538 and 1681 hits, respectively. The occurrence of multiple BLAST hits was considered to reflect paralogs or intron spanning sequences. These were removed from the initial collection of SNPs that were first developed for GoldenGate analysis (Anithakumari et al. 2010). This resulted in 279 and 453 unique BLAST hits representing the occurrence of only one copy in the potato genome sequence of *Solanum tuberosum* Group Phureja DM1-3 516R44 (CIP801092) as known hitherto (Potato Genome Sequencing Consortium, www.potatogemome.net).

From the total of 732 SNPs with unique BLAST hits only a few (31 cases, equally distributed over the 384 and 768 set) were omitted due to high level of sequence mismatches (3-6 SNPs within 101 bp). One mismatch should be expected and a second SNP was tolerated to be included in the SNP-spanning sequences selected.

For the present SNP design the minimal sequence length was set at 101 bp. Therefore 81 sequences were removed as they were shorter. The resulting 553 sequences have a length of at least 101 bp. A total of 100 sequences were then removed for having incomplete matches of only 35 up to 94 nucleotides within a scaffold of a doubled monoploid (DM-3 516R44; www.potatogenome.net). Also removed were sequences with introduced alignment gaps and sequences having other potential mismatch problems.

Though twenty-five sequences did not meet the criteria described above, they were kept as these were were known to be highly informative in the GoldenGate SNP assays. These SNPs even allowed to discriminate between the five genotype classes that occur in tetraploids (Voorrips et al, EAPR-EUCARPIA congress "Potato Breeding after completion of the DNA Sequence of the Potato Genome", 27-30 June 2010, Wageningen, the Netherlands, e-publication at http://edepot.wur.nl/143559, pg 42).

Finally, 515 sequences were selected. The final selection of 100 sequences was based on the known map position of 237 markers (out of the 515) on the potato genetic map (Anithakumari et al, 2010 and Van Os et al. Genetics 173: 1075-1087, 2006). The selected markers were evenly distributed over the 12 potato chromosomes including the most distal telomeric as well as centromeric markers and with a preference for SNPs that were heterozygous in clone RH (RH89-039-16; www.potatogenome.net), because clone RH is a progenitor of the material used in the present research. Or in other words, the alleles of clone RH are also present in material used in the present research (see Table 2 and Figure 3).

Testing of markers

The 100 SNP sequences selected as described above were used in a KASPar SNP genotyping system (KBioscience Ltd. Hoddesdon, UK; http://www.kbioscience.co.uk/lab%20services/SNP%20Genotyping/genotyping_chemistry.html). The markers were tested on a set of 20 diploid potato genotypes (see Figure 4) including the three diploid parents used in the present study and further on the donor of the *Sli* gene, clone 07-1004-1 (see also Example 2 herein above).

Results

All SNP sequences generated polymorphisms in the KASPar SNP genotyping system (see Figure 4), corresponding to a 100% success rate. In total, 100 markers x 20 genotypes were tested, comprising 2000 datapoints. We observed 29 missing data, which is less than 2 %. As control, one genotype was tested twice whereby plant samples from different locations were used (Figure 4, columns 4 and 7). All data were identical. These results illustrate that the KASPar SNP genotyping system is an efficient, reliable and reproducible marker platform for diploid potato. This indicated that The KASPar SNP genotyping system is efficient, reliable and reproducible for diploid potato.

All markers showed a high frequency of occurrence in the diploid potato germplasm used in this experiment (Appendix III). The heterozygote frequency in the germplasm by suing these 100 markers ranged from 26 % to 77%, while the *Sli* gene donor was 100% homozygous as is in accordance with expectations (Phumichai et al. 2005 Genome 48: 977–984).

The number of informative markers between the Sli donor and the three parents used in the present study was 54, 52 and 62 resp. This indicates that the KASPar SNP genotyping system is also informative for diploid potato.

Based on the above, it was concluded that the KASPar SNP genotyping system as described herein and based on the markers as developed herein is efficient, reliable and reproducible and very informative to describe the level of homozygosity / heterezygosity in diploid potato.

Table 5A. Sequences of 100 SNP markers in potato

SNPs

SNPID	SNPNum	AlleleY	AlleleX	Sequence
PotSNP002	11940065	G	C	ACCTGGTGCAGGCCT[C/G]CGTATTGATACGATT
PotSNP011	11940098	C	T	GACTGTGATTCGAAA[T/C]GTTTACCTATCTCTT
PotSNP021	11940075	C	T	TCGTAGAAATGACAT[T/C]TGGGGTGCTAGAGAT
PotSNP026	11940044	C	T	AGTTTCCAGCTGATG[T/C]TATTCTAGGAGACGA
PotSNP032	11940037	G	A	AGAGGCTCTTGAGGC[A/G]CAGCGAAAAGAGGAA
PotSNp038	11940016	C	T	TGGTGACGTTCATGT[T/C]GGACGTAATGCTTCT
PotSNP043	11940036	C	A	AGTATCTCCGTCCGA[A/C]TCACAGTCCTACTCT
PotSNP045	11940094	G	A	GGTGCTAAGATTCGC[A/G]TTGATGATTCACTAG
PotSNP047	11940054	C	T	CCAACACTGGAAAAA[T/C]TATGGAAGCAGAAAG
PotSNP052	11940097	C	T	GCTAGCATTAGAGCT[T/C]GCTAAGCGACTCAAT
PotSNP055	11940021	G	A	GCCTTGGAGTAAGAG[A/G]TTTTCTGTTTTGGGC
PotSNP057	11940040	C	T	GGATGTTCATCAACG[T/C]CGACTTCGACAAGCT
PotSNP061	11940032	C	T	ACAACCTGCAAGAGC[T/C]TTGCATCTGTTTAGT
PotSNP068	11940023	C	T	TACAATCATGATCCT[T/C]TTGCCTAATTGCCCT
PotSNP072	11940005	G	A	CTGAGCTAGTAGATC[A/G]ATCAAACGGTGCTCC
PotSNP073	11940076	G	T	GACTGTATGGACAGT[T/G]ATTGGGTCAGAGGAC
PotSNP080	11940068	C	T	AAGATGACGTGGAGT[T/C]CGGCACTGCGCCGTT
PotSNP081	11940047	C	T	GCCGCACAAGGTGCT[T/C]GCCGTCGATGTTAGC
PotSNP083	11940033	C	T	AGGTAGTGTTGCAAA[T/C]ACAATTAGAGGGTTG
PotSNP089	11940062	G	A	AAGTACATTTATGAG[A/G]AGATTAACAAGTGTT
PotSNP099	11940092	C	T	ACTGCTGGAACTGAG[T/C]TGAACAATGCTATTA
PotSNP100	11940083	C	T	GAGTGCCGGCAGCGT[T/C]ACAGATCCGACGATG
PotSNP102	11940070	G	A	TATGGCAGAGAAAGA[A/G]ATGGAGTATAGAGTG
PotSNP1026	11940059	G	C	TAAACTCCAACATAT[C/G]AACACAAATTAGTGC
PotSNP1034	11940001	G	A	TTCTACATCCTTCGC[A/G]GGCATTTTTGAGGAG
PotSNP1049	11940051	T	C	TTTGGGGAGGAACAC[C/T]TGTGCTTTAGAACTC
PotSNP1057	11940022	A	C	TGTCACTAAGAAAGC[C/A]CAAGAGGCAATGCAG
PotSNP106	11940010	G	T	GCAAGCTACTTGTTC[T/G]AAAGGACCTGCTACT
PotSNP1072	11940027	G	T	AGCGAGTTTGGATGC[T/G]GAGCTTTTGCAGTTA
PotSNP1083	11940019	C	T	TACAAAGTTCACTGC[T/C]GAGGAGGTCATGCAG
PotSNP1105	11940041	A	C	GATGAGGAAAGGCAC[C/A]GGCGGAGGTGTTGGA
PotSNP1115	11940071	T	C	CACCACCGTCCAAAC[C/T]GGTTTTGCCAATCGC
PotSNP1122	11940060	T	C	GGTCCCTGAGCAAGG[C/T]AATCAACGAATTAGG
PotSNP1139	11940004	G	A	AGTCTCTACAAACCC[A/G]TGGTTTCGATCTTGA
PotSNP116	11940058	G	A	TAATGTGGTCGGCAC[A/G]CTGACGCTTGCTGAT
PotSNP118	11940091	G	A	AGATGCATTCTATGC[A/G]GAACCACAAAGATAT
PotSNP120	11940082	G	A	TTCGTGTTGCACTCA[A/G]CGATTCAGTTGGATG
PotSNP121	11940099	C	T	AGCTTGTGCTAATAG[T/C]TGTGCTGATTTTCTC
PotSNP122	11940078	G	A	TATGCAATCCCAGCC[A/G]ACATATCCGATCCAT
PotSNP124	11940055	G	A	TCATACTTCACATAT[A/G]TCAGAGTGGTCTGAT
PotSNP130	11940050	C	T	AGAGAAAAAAGCTGT[T/C]GTTGTTGATGACGAG
PotSNP134	11940080	C	T	CGCGTATTTCCCTTA[T/C]TATAACTCGTTCTCT
PotSNP138	11940052	C	T	TTACATATGCAATAG[T/C]GGTGGTGATCTTTAT

PotSNP152	11940077	C	T	GAAGTTGTCTTACAT[T/C]GCTCTTGACTATGAA
PotSNP162	11940074	C	T	CTATGATGCATTTAG[T/C]TCATGGAGCAAACTT
PotSNP165	11940009	G	A	AGTTCCTTTTTCTCC[A/G]AGTTCTCAGACTCCG
PotSNP178	11940026	G	T	GAGGAATTGATGCGT[T/G]CTGAACTGGCACAGT
PotSNP182	11940095	G	A	ATACACAGGAAGGGT[A/G]AAGTGCTTCAAATTG
PotSNP184	11940038	G	A	TTTGACCATTCTTTC[A/G]GTCCTAGCTAACCAC
PotSNP185	11940002	C	T	AAGATAAGAAGAAAG[T/C]TAAGAGAACCAAGAT
PotSNP194	11940064	C	T	GGCCCACTTTCCACA[T/C]ATTCAATTTGGTCTC
PotSNP205	11940042	G	A	TCAACTCACCACTGG[A/G]TTTTACTCAAAATCA
PotSNP213	11940096	G	A	TAATATGTTGTGTCT[A/G]TAATTTTTGGGTCAT
PotSNP220	11940013	C	T	CATTAACCAAGGTTG[T/C]ATCGTTCGAGAGATC
PotSNP229	11940056	C	T	TCCTTTGTCATTGTA[T/C]GCCATGGAAGAAGCA
PotSNP392	11940003	T	A	GCTTTGTTTAAGAAC[A/T]GCCAGAAGAAGTTGC
PotSNP401	11940012	G	A	GCCTACGCGGCATGA[A/G]GATGTGGATATTGTT
PotSNP402	11940072	T	C	AAAGCTCAAATTAAA[C/T]GACGATGTTCAGGAA
PotSNP423	11940020	A	G	GGCCAACCAATTCCA[G/A]CGTCTTCTCCCAGAC
PotSNP446	11940081	C	T	AAAACCATTTGGTCT[T/C]GTTGATAATTCTGAG
PotSNP458	11940069	A	G	TACATAGCTCTACAA[G/A]CTTGGAAACATGTCA
PotSNP470	11940043	T	A	GACACAAAATTCATA[A/T]TGGCTGATACTTTGT
PotSNP543	11940049	T	A	GCAACTTGTCGTCGG[A/T]AAACTCGACTAGATT
PotSNP567	11940014	A	C	CTAGAAACAAAATCA[C/A]AATATAAGTTACATA
PotSNP569	11940028	G	A	GTTGTTTGCTTTGTT[A/G]TGCTGACTTATTGTA
PotSNP573	11940034	C	T	ATGCTTCTTGGTTGC[T/C]GTTTGTTTAGGCTCT
PotSNP580	11940087	T	C	AAATATGTTGTTCCA[C/T]TAGAAAAAAGAGTAA
PotSNP586	11940088	C	T	GTGAATACTGGTATC[T/C]TTCCAATTGAAGTCA
PotSNP587	11940067	C	T	AATATTCTTGTAGTA[T/C]ATATTGTCTAAAATC
PotSNP607	11940084	T	C	CAACGATATCGAGAA[C/T]TATTGTTCTAAGGGA
PotSNP626	11940045	G	A	GCTGAAAGTAAAGAC[A/G]GTGTTCAACATCTTG
PotSNP645	11940007	T	G	CATGCAATTGTAATA[G/T]TATCAATGTTTGTTG
PotSNP652	11940024	T	C	TGCTCAAGCTGCCAA[C/T]GCTAATCCGCTTTAC
PotSNP700	11940061	G	A	CAAGATATATGCAGC[A/G]GAGTCCCTTCGAGCA
PotSNP702	11940039	A	T	TTATGGTTATGGTGG[T/A]GGAGGTTGGATTAAT
PotSNP712	11940057	C	G	GGGAGGGTGAAAATA[G/C]TCGAAAACGGGCAAC
PotSNP713	11940073	G	A	GATTGTTATCCTTCC[A/G]CCACCTCCAACAGAT
PotSNP731	11940035	A	G	CAACTTCCAGTCGTG[G/A]GAGTGGGGTTAACGG
PotSNP753	11940046	G	A	ATGGTGTCTCCAAGG[A/G]ACTATTACAGTCACT
PotSNP759	11940090	C	T	AACATCGATAGGACT[T/C]TGAAGGCCGCGGATG
PotSNP766	11940089	T	C	CTGCCACTGCGGCTG[C/T]AGGTTCTGCAGCTGC
PotSNP775	11940079	G	A	TGAAATGATTGGATT[A/G]TGACCCATAGTTTAG
PotSNP796	11940025	C	A	ATCTCTAAGTTGGGG[A/C]AACCCATAATTTCTA
PotSNP827	11940053	T	A	CGCTTGGGAAGAACC[A/T]TTCGGTCCAGTTTTG
PotSNP834	11940006	T	C	CCAATTCCGACGGCC[C/T]TCGTCGTTTCACCGC
PotSNP853	11940066	A	C	GGTCGACCATTGGGG[C/A]CCCGTAGAGGGTATA
PotSNP866	11940048	G	T	AAGGCGAATTCCGGT[T/G]CCCGAAATGGATCTC
PotSNP893	11940011	A	G	TCATTACATCAAAAT[G/A]TTTTATCAATGCCCA
PotSNP908	11940100	T	A	TGATCCATTTGGTCT[A/T]GACCAGTTCTTGACA
PotSNP910	11940017	T	A	TGCTGTGCTCCTTCT[A/T]TTCCTCATCTCTCAC

PotSNP928	11940030	A	T	TCATTCTCACTCTCC[T/A]GCTGAACAAGCCATT
PotSNP947	11940015	T	G	AAAAGACAAGAATCT[G/T]GTTCAGATTCTCCAG
PotSNP948	11940063	T	A	TGGGGAAAACTTAAT[A/T]TGACAGCCAAGGCGT
PotSNP960	11940031	C	T	GGTTTTGTGGACTGC[T/C]AACACTGAAAGATAC
PotSNP964	11940029	A	T	TTATTTCAATGAAGC[T/A]TCTGGTGGACGTTAT
PotSNP982	11940085	C	T	TCTGTGGTTTTTTCA[T/C]CACCTCCATCTTCAA
PotSNP983	11940086	G	A	TAAGATTAGTTCTAA[A/G]CTTAGCGCAGAAGAC
PotSNP985	11940018	A	G	GAGCTCTATAAGTGC[G/A]CCTGTTACTCCACCT
PotSNP987	11940008	C	A	GGGCTATGGTTGGTT[A/C]GGAAAAAAGGTGCAA
PotSNP996	11940093	C	T	GGATATTGGGTTCGT[T/C]CCATGATTCGTGGCA

Table 5A. Sequences of 100 SNP markers in potato

SNPs					Sequence
SNPID	SNPNum	AlleleY	AlleleX	Sequence	Identifier
PotSNP002	11940065	G	C	ACCTGGTGCAGGCCT [C/G] CGTATTGATACGATT	SEQ.ID NO:1
PotSNP011	11940098	C	T	GACTGTGATTCGAAA [T/C] GTTTACCTATCTCTT	SEQ.ID NO:2
PotSNP021	11940075	C	T	TCGTAGAAATGACAT [T/C] TGGGGTGCTAGAGAT	SEQ.ID NO:3
PotSNP026	11940044	C	T	AGTTTCCAGCTGATG [T/C] TATTCTAGGAGACGA	SEQ.ID NO:4
PotSNP032	11940037	G	A	AGAGGCTCTTGAGGC [A/G] CAGCGAAAAGAGGAA	SEQ.ID NO:5
PotSNp038	11940016	C	T	TGGTGACGTTCATGT [T/C] GGACGTAATGCTTCT	SEQ.ID NO:6
PotSNP043	11940036	C	A	AGTATCTCCGTCCGA [A/C] TCACAGTCCTACTCT	SEQ.ID NO:7
PotSNP045	11940094	G	A	GGTGCTAAGATTCGC [A/G] TTGATGATTCACTAG	SEQ.ID NO:8
PotSNP047	11940054	C	T	CCAACACTGGAAAAA [T/C] TATGGAAGCAGAAAG	SEQ.ID NO:9
PotSNP052	11940097	C	T	GCTAGCATTAGAGCT [T/C] GCTAAGCGACTCAAT	SEQ.ID NO:10
PotSNP055	11940021	G	A	GCCTTGGAGTAAGAG [A/G] TTTTCTGTTTTGGGC	SEQ.ID NO:11
PotSNP057	11940040	C	T	GGATGTTCATCAACG [T/C] CGACTTCGACAAGCT	SEQ.ID NO:12
PotSNP061	11940032	C	T	ACAACCTGCAAGAGC [T/C] TTGCATCTGTTTAGT	SEQ.ID NO:13
PotSNP068	11940023	C	T	TACAATCATGATCCT [T/C] TTGCCTAATTGCCCT	SEQ.ID NO:14
PotSNP072	11940005	G	A	CTGAGCTAGTAGATC [A/G] ATCAAACGGTGCTCC	SEQ.ID NO:15
PotSNP073	11940076	G	T	GACTGTATGGACAGT [T/G] ATTGGGTCAGAGGAC	SEQ.ID NO:16

PotSNP080	11940068	C	T	AAGATGACGTGGAGT [T/C] CGGCACTGCGCCGTT	SEQ.ID NO:17
PotSNP081	11940047	C	T	GCCGCACAAGGTGCT [T/C] GCCGTCGATGTTAGC	SEQ.ID NO:18
PotSNP083	11940033	C	T	AGGTAGTGTTGCAAA [T/C] ACAATTAGAGGGTTG	SEQ.ID NO:19
PotSNP089	11940062	G	A	AAGTACATTTATGAG [A/G] AGATTAACAAGTGTT	SEQ.ID NO:20
PotSNP099	11940092	C	T	ACTGCTGGAACTGAG [T/C] TGAACAATGCTATTA	SEQ.ID NO:21
PotSNP100	11940083	C	T	GAGTGCCGGCAGCGT [T/C] ACAGATCCGACGATG	SEQ.ID NO:22
PotSNP102	11940070	G	A	TATGGCAGAGAAAGA [A/G] ATGGAGTATAGAGTG	SEQ.ID NO:23
PotSNP1026	11940059	G	C	TAAACTCCAACATAT [C/G] AACACAAATTAGTGC	SEQ.ID NO:24
PotSNP1034	11940001	G	A	TTCTACATCCTTCGC [A/G] GGCATTTTTGAGGAG	SEQ.ID NO:25
PotSNP1049	11940051	T	C	TTTGGGGAGGAACAC [C/T] TGTGCTTTAGAACTC	SEQ.ID NO:26
PotSNP1057	11940022	A	C	TGTCACTAAGAAAGC [C/A] CAAGAGGCAATGCAG	SEQ.ID NO:27
PotSNP106	11940010	G	T	GCAAGCTACTTGTTC [T/G] AAAGGACCTGCTACT	SEQ.ID NO:28
PotSNP1072	11940027	G	T	AGCGAGTTTGGATGC [T/G] GAGCTTTTGCAGTTA	SEQ.ID NO:29
PotSNP1083	11940019	C	T	TACAAAGTTCACTGC [T/C] GAGGAGGTCATGCAG	SEQ.ID NO:30
PotSNP1105	11940041	A	C	GATGAGGAAAGGCAC [C/A] GGCGGAGGTGTTGGA	SEQ.ID NO:31
PotSNP1115	11940071	T	C	CACCACCGTCCAAAC [C/T] GGTTTTGCCAATCGC	SEQ.ID NO:32
PotSNP1122	11940060	T	C	GGTCCCTGAGCAAGG [C/T] AATCAACGAATTAGG	SEQ.ID NO:33
PotSNP1139	11940004	G	A	AGTCTCTACAAACCC [A/G] TGGTTTCGATCTTGA	SEQ.ID NO:34
PotSNP116	11940058	G	A	TAATGTGGTCGGCAC[A/G] CTGACGCTTGCTGAT	SEQ.ID NO:35
PotSNP118	11940091	G	A	AGATGCATTCTATGC [A/G] GAACCACAAAGATAT	SEQ.ID NO:36
PotSNP120	11940082	G	A	TTCGTGTTGCACTCA [A/G] CGATTCAGTTGGATG	SEQ.ID NO:37
PotSNP121	11940099	C	T	AGCTTGTGCTAATAG [T/C] TGTGCTGATTTTCTC	SEQ.ID NO:38
PotSNP122	11940078	G	A	TATGCAATCCCAGCC [A/G] ACATATCCGATCCAT	SEQ.ID NO:39

PotSNP124	11940055	G	A	TCATACTTCACATAT [A/G] TCAGAGTGGTCTGAT	SEQ.ID NO:40
PotSNP130	11940050	C	T	AGAGAAAAAAGCTGT [T/C] GTTGTTGATGACGAG	SEQ.ID NO:41
PotSNP134	11940080	C	T	CGCGTATTTCCCTTA [T/C] TATAACTCGTTCTCT	SEQ.ID NO:42
PotSNP138	11940052	C	T	TTACATATGCAATAG [T/C] GGTGGTGATCTTTAT	SEQ.ID NO:43
PotSNP152	11940077	C	T	GAAGTTGTCTTACAT [T/C] GCTCTTGACTATGAA	SEQ.ID NO:44
PotSNP162	11940074	C	T	CTATGATGCATTTAG [T/C] TCATGGAGCAAACTT	SEQ.ID NO:45
PotSNP165	11940009	G	A	AGTTCCTTTTTCTCC [A/G] AGTTCTCAGACTCCG	SEQ.ID NO:46
PotSNP178	11940026	G	T	GAGGAATTGATGCGT [T/G] CTGAACTGGCACAGT	SEQ.ID NO:47
PotSNP182	11940095	G	A	ATACACAGGAAGGGT [A/G] AAGTGCTTCAAATTG	SEQ.ID NO:48
PotSNP184	11940038	G	A	TTTGACCATTCTTTC [A/G] GTCCTAGCTAACCAC	SEQ.ID NO:49
PotSNP185	11940002	C	T	AAGATAAGAAGAAAG [T/C] TAAGAGAACCAAGAT	SEQ.ID NO:50
PotSNP194	11940064	C	T	GGCCCACTTTCCACA [T/C] ATTCAATTTGGTCTC	SEQ.ID NO:51
PotSNP205	11940042	G	A	TCAACTCACCACTGG [A/G] TTTTACTCAAAATCA	SEQ.ID NO:52
PotSNP213	11940096	G	A	TAATATGTTGTGTCT [A/G] TAATTTTTGGGTCAT	SEQ.ID NO:53
PotSNP220	11940013	C	T	CATTAACCAAGGTTG [T/C] ATCGTTCGAGAGATC	SEQ.ID NO:54
PotSNP229	11940056	C	T	TCCTTTGTCATTGTA [T/C] GCCATGGAAGAAGCA	SEQ.ID NO:55
PotSNP392	11940003	T	A	GCTTTGTTTAAGAAC [A/T] GCCAGAAGAAGTTGC	SEQ.ID NO:56
PotSNP401	11940012	G	A	GCCTACGCGGCATGA [A/G] GATGTGGATATTGTT	SEQ.ID NO:57
PotSNP402	11940072	T	C	AAAGCTCAAATTAAA [C/T] GACGATGTTCAGGAA	SEQ.ID NO:58
PotSNP423	11940020	A	G	GGCCAACCAATTCCA [G/A] CGTCTTCTCCCAGAC	SEQ.ID NO:59
PotSNP446	11940081	C	T	AAAACCATTTGGTCT [T/C] GTTGATAATTCTGAG	SEQ.ID NO:60
PotSNP458	11940069	A	G	TACATAGCTCTACAA [G/A] CTTGGAAACATGTCA	SEQ.ID NO:61
PotSNP470	11940043	T	A	GACACAAAATTCATA [A/T] TGGCTGATACTTTGT	SEQ.ID NO:62
PotSNP543	11940049	T	A	GCAACTTGTCGTCGG [A/T] AAACTCGACTAGATT	SEQ.ID NO:63

PotSNP567	11940014	A	C	CTAGAAACAAAATCA [C/A] AATATAAGTTACATA	SEQ.ID NO:64
PotSNP569	11940028	G	A	GTTGTTTGCTTTGTT [A/G] TGCTGACTTATTGTA	SEQ.ID NO:65
PotSNP573	11940034	C	T	ATGCTTCTTGGTTGC [T/C] GTTTGTTTAGGCTCT	SEQ.ID NO:66
PotSNP580	11940087	T	C	AAATATGTTGTTCCA [C/T] TAGAAAAAAGAGTAA	SEQ.ID NO:67
PotSNP586	11940088	C	T	GTGAATACTGGTATC [T/C] TTCCAATTGAAGTCA	SEQ.ID NO:68
PotSNP587	11940067	C	T	AATATTCTTGTAGTA [T/C] ATATTGTCTAAAATC	SEQ.ID NO:69
PotSNP607	11940084	T	C	CAACGATATCGAGAA [C/T] TATTGTTCTAAGGGA	SEQ.ID NO:70
PotSNP626	11940045	G	A	GCTGAAAGTAAAGAC [A/G] GTGTTCAACATCTTG	SEQ.ID NO:71
PotSNP645	11940007	T	G	CATGCAATTGTAATA [G/T] TATCAATGTTTGTTG	SEQ.ID NO:72
PotSNP652	11940024	T	C	TGCTCAAGCTGCCAA [C/T] GCTAATCCGCTTTAC	SEQ.ID NO:73
PotSNP700	11940061	G	A	CAAGATATATGCAGC [A/G] GAGTCCCTTCGAGCA	SEQ.ID NO:74
PotSNP702	11940039	A	T	TTATGGTTATGGTGG [T/A] GGAGGTTGGATTAAT	SEQ.ID NO:75
PotSNP712	11940057	C	G	GGGAGGGTGAAAATA[G/C] TCGAAAACGGGCAAC	SEQ.ID NO:76
PotSNP713	11940073	G	A	GATTGTTATCCTTCC [A/G] CCACCTCCAACAGAT	SEQ.ID NO:77
PotSNP731	11940035	A	G	CAACTTCCAGTCGTG [G/A] GAGTGGGGTTAACGG	SEQ.ID NO:78
PotSNP753	11940046	G	A	ATGGTGTCTCCAAGG [A/G] ACTATTACAGTCACT	SEQ.ID NO:79
PotSNP759	11940090	C	T	AACATCGATAGGACT [T/C] TGAAGGCCGCGGATG	SEQ.ID NO:80
PotSNP766	11940089	T	C	CTGCCACTGCGGCTG [C/T] AGGTTCTGCAGCTGC	SEQ.ID NO:81
PotSNP775	11940079	G	A	TGAAATGATTGGATT [A/G] TGACCCATAGTTTAG	SEQ.ID NO:82
PotSNP796	11940025	C	A	ATCTCTAAGTTGGGG [A/C] AACCCATAATTTCTA	SEQ.ID NO:83
PotSNP827	11940053	T	A	CGCTTGGGAAGAACC [A/T] TTCGGTCCAGTTTTG	SEQ.ID NO:84
PotSNP834	11940006	T	C	CCAATTCCGACGGCC [C/T] TCGTCGTTTCACCGC	SEQ.ID NO:85
PotSNP853	11940066	A	C	GGTCGACCATTGGGG[C/A] CCCGTAGAGGGTATA	SEQ.ID NO:86
PotSNP866	11940048	G	T	AAGGCGAATTCCGGT [T/G] CCCGAAATGGATCTC	SEQ.ID NO:87
PotSNP893	11940011	A	G	TCATTACATCAAAAT [G/A] TTTTATCAATGCCCA	SEQ.ID NO:88

PotSNP908	11940100	T	A	TGATCCATTTGGTCT [A/T] GACCAGTTCTTGACA	SEQ.ID NO:89
PotSNP910	11940017	T	A	TGCTGTGCTCCTTCT [A/T] TTCCTCATCTCTCAC	SEQ.ID NO:90
PotSNP928	11940030	A	T	TCATTCTCACTCTCC [T/A] GCTGAACAAGCCATT	SEQ.ID NO:91
PotSNP947	11940015	T	G	AAAAGACAAGAATCT [G/T] GTTCAGATTCTCCAG	SEQ.ID NO:92
PotSNP948	11940063	T	A	TGGGGAAAACTTAAT [A/T] TGACAGCCAAGGCGT	SEQ.ID NO:93
PotSNP960	11940031	C	T	GGTTTTGTGGACTGC [T/C] AACACTGAAAGATAC	SEQ.ID NO:94
PotSNP964	11940029	A	T	TTATTTCAATGAAGC [T/A] TCTGGTGGACGTTAT	SEQ.ID NO:95
PotSNP982	11940085	C	T	TCTGTGGTTTTTTCA [T/C] CACCTCCATCTTCAA	SEQ.ID NO:96
PotSNP983	11940086	G	A	TAAGATTAGTTCTAA [A/G] CTTAGCGCAGAAGAC	SEQ.ID NO:97
PotSNP985	11940018	A	G	GAGCTCTATAAGTGC [G/A] CCTGTTACTCCACCT	SEQ.ID NO:98
PotSNP987	11940008	C	A	GGGCTATGGTTGGTT [A/C] GGAAAAAAGGTGCAA	SEQ.ID NO:99
PotSNP996	11940093	C	T	GGATATTGGGTTCGT [T/C] CCATGATTCGTGGCA	SEQ.ID NO:100

Table 5B: List of PotSNPmarkers and their specific annotation for position on the SH and RH map of Figure 3.

Marker	Annotation	Marker	Annotation	Marker	Annotation
PotSNP072	1.1	PotSNP573	5.1	PotSNP102	9.1
PotSNP834	1.2	PotSNP184	5.2	PotSNP587	9.2
PotSNP645	1.3	PotSNP702	5.3	PotSNP1115	9.3
PotSNP1034	1.4	PotSNP731	5.4	PotSNP402	9.4
PotSNP987	1.5	PotSNP043	5.5	PotSNP713	9.5
PotSNP185	1.6	PotSNP057	5.6	PotSNP080	9.6
PotSNP392	1.7	PotSNP1105	5.7	PotSNP458	9.7
PotSNP165	1.8	PotSNP032	5.8	PotSNP162	9.8
PotSNP1139	1.9	PotSNP205	6.1	PotSNP021	10.1
PotSNP038	2.1	PotSNP753	6.2	PotSNP073	10.2
PotSNP910	2.2	PotSNP470	6.3	PotSNP152	10.3
PotSNP106	2.3	PotSNP026	6.4	PotSNP122	10.4
PotSNP893	2.4	PotSNP626	6.5	PotSNP775	10.5
PotSNP401	2.5	PotSNP081	6.6	PotSNP134	10.6
PotSNP220	2.6	PotSNP866	6.7	PotSNP446	10.7
PotSNP567	2.7	PotSNP124	7.1	PotSNP120	10.8
PotSNP947	2.8	PotSNP047	7.2	PotSNP586	11.1
PotSNP055	3.1	PotSNP543	7.3	PotSNP100	11.2
PotSNP985	3.2	PotSNP130	7.4	PotSNP607	11.3

PotSNP1083	3.3	PotSNP1049	7.5	PotSNP766	11.4
PotSNP423	3.4	PotSNP229	7.6	PotSNP759	11.5
PotSNP1057	3.5	PotSNP712	7.7	PotSNP118	11.6
PotSNP068	3.6	PotSNP138	7.8	PotSNP982	11.7
PotSNP652	3.7	PotSNP827	7.9	PotSNP983	11.8
PotSNP796	3.8	PotSNP1026	8.1	PotSNP580	11.9
PotSNP178	4.1	PotSNP1122	8.2	PotSNP099	12.1
PotSNP1072	4.2	PotSNP700	8.3	PotSNP011	12.2
PotSNP960	4.3	PotSNP853	8.4	PotSNP996	12.3
PotSNP061	4.4	PotSNP089	8.5	PotSNP045	12.4
PotSNP569	4.5	PotSNP948	8.6	PotSNP121	12.5
PotSNP083	4.6	PotSNP194	8.7	PotSNP908	12.6
PotSNP964	4.7	PotSNP116	8.8	PotSNP182	12.7
PotSNP928	4.8	PotSNP002	8.9	PotSNP213	12.8
				PotSNP052	12.9

Table 6. Diploid potato genotypes used for SNP marker development and testing

Nr.	Abbr.	code	Short description	reference
1	H	07-1004-1	*Sli* gene donor	07-1004-1 is a progeny of the *Sli*-donor F1-1 as described in Phumichai et al. (2005) Genome Vol. 48:977-984, Phumichai and Hosaka (2006) Euphytica 149:251–258 and Phumichai et al. (2006) Euphytica 148: 227–234.
2	D1	IVP97-079-9	Parent 1 used in present research, early, long, Y (yellow flesh), Qcook	Examples 1, 2 and 4 herein; available from Ronald Hutten, Laboratory for Plant Breeding, Wageningen University.
3	D2	IVPAA-096-18	Parent 2 used in present research, early, Y, Qcook	Examples 1, 2 and 4 herein; available from Ronald Hutten, Laboratory for Plant Breeding, Wageningen University.
4	D3	SH83-92-488	Parent 3 used in present research, *R3*, *H1*, *Gpa2*, *RXadg*, Y	Examples 2 and 4 herein; available from Ronald Hutten, Laboratory for Plant Breeding, Wageningen University.
5	D4	parent C	Parent of the CxE population	Anithakumari A.M. et al. (2010) Molecular Breeding 26(1):65-75
6	D5	parent E	Parent of the CxE population	Anithakumari A.M. et al. (2010) Molecular Breeding 26(1):65-75
7	D3	SH83-92-488	Independent duplo of nr 3	Van Os, H. et al. (2006) Genetics 173(2):1075-1087
8	D6	RH89-039-16	Used for genome sequencing	Van Os, H. et al. (2006) Genetics 173(2):1075-1087; available from Ronald Hutten, Laboratory for Plant Breeding, Wageningen University
9	D7	3778-16	*Grp1*, early, long (shape), *Ro1*	Rouppe van der Voort et al. (1997) Mol Gen Genetics 255(4):438-447
10	D8	IVP92-057-3	early, long, Y, Qfry	Used for marker development in Example 3
11	D9	IVP98-082-14	Long, Y, Qfry, *H1*, Qcook	Used for marker development in Example 3
12	D10	IVP02-089-5	Early, long, Y, *H1*, Qcook, Zep (orange flesh)	Used for marker development in Example 3
13	D11	IVP05-113-1	Early, y(white flesh)	Used for marker development in Example 3
14	D12	IVP05-122-24	Qstarch, Y	Used for marker development in Example 3
15	D13	IVP06-142-12	Wild species hybrid: Phyt avl	Used for marker development in Example 3
16	D14	IVP06-145-2	Wild species hybrid: Phyt rch	Used for marker development in Example 3

17	D15	IVP06-148-13	Round (shape), Qcook, Qfry	Used for marker development in Example 3
18	D16	IVP06-149-12	Early, round, Zep, Y, spectacled, Qcook, blue anthocyans	Used for marker development in Example 3
19	D17	IVP06-155-9	Wild species hybrid: Phyt tar	Used for marker development in Example 3
20	D18	IVP06-161-16	Early, Phyt vnt1, round, Y, *H1*	Used for marker development in Example 3
21	D19	IVPAA-134-16	Early, round, y (white flesh)	Used for marker development in Example 3

Early: early flowering (short cycle); Qcook: good cooking quality; Qfry: good frying quality; Phyt indicates *Phytophthera* resistance genes

Example 4. Development of homozygous diploid potato plants and generation of heterozygous F1 hybrid progeny

Introduction

The objectives of this Example were to develop homozygous diploid potato lines that are self-compatible (SC) and have good agronomic performance. A breeding programme to combine self-compatibility with good agronomic performance is described above in Example 2 above. The aim of the current experiment is to prove that homozygous diploid self-compatible potato genotypes can be generated by inbreeding and that diploid potato plants containing contrasting homozygous loci scattered over the potato genome can be crossed (are intercrossable) to generate heterozygous F1 hybrid progeny.

Materials and methods

A self-compatible diploid homozygous potato genotype, that harbours the *Sli*-gene as described in the Examples above was crossed with two potato clones, designated D1 and D2 (see Examples 1 and 2) The F1 progenies were selfed and two F2 progenies were generated from two random SC F1 plants. Random self-compatible plants of these two F2 populations were selfed and per F2 population three F3 populations were tested for level of homozygosity and for segregation of SNP markers.

The 6 F2 plants were tested for ploidy level (by fluorescent quantitation of DNA) and were all found to be diploid.

A set of 36 markers was used on 265 individual F3 plants by applying the KASPar SNP genotyping system (see Example 3) covering all 24 chromosome arms of potato (see Figure 5).

Results

Subsets of 35 to 56 plants from the six F3 populations, in total 265 plants, were tested with at least 24 informative markers per parent pair. In total 36 markers were used (see Figure 5). For position of markers on the potato genome see Example 3, above. The *Sli* donor was 100% homozygous for these markers tested, while the D1 and D2 parents contained 20 and 19 loci resp. that were heterozygous out of the 24 informative markers tested per parent pair, while the other five and six loci resp. contained homozygous alleles originating from the from the donor parent (D1 or D2).

Six homozygous F3 plants

As expected, the F3 populations segregated for markers that were heterozygous in the F2 and not for markers that were fixed in the F2 (Figure 5). The mean heterozygote level ranged from 84 to 95 %, while that of individual plants ranged from 71 to 100% (Table 7).

Table 7. Homozygote frequencies (%) of six F3 populations

F3 population	Donor parent	mean	range
126	D1	85	74 – 96
126B	D1	88	74 – 100
127	D1	84	71 – 96
121	D2	85	71 - 96
122	D2	89	79 -100
123	D2	94	88 - 100

Three F3 populations of the D1 x H and of the H x D2 crosses were analysed for frequency of homozygous loci based on the data in Figure 5 as shown in Table 9.

These frequencies are expected giving the allele frequencies of the parents. There were six plants from three populations that were already 100 % homozygous. These harboured mainly homozygous loci with *Sli* donor alleles. This is expected as the *Sli* donor harbours homozygous alleles, while the other parents contained one common allele with the *Sli* donor per heterozygous marker. So, *Sli*–donor derived alleles were abundant in all offspring populations. These six homozygous F3 plants, designated 122-34, 123-6, 123-20,

123-23, 123-24 and 126B-17, contained two, five, four, three, five and seven loci resp. with homozygous alleles from the donor parent. So, already in two selfed generations 100 % homozygosity was reached for 24 marker loci representing 24 chromosome arms.

No absolute lethal allele

It is often stated that repeated selfings in diploid potato will only result in weak plants due to inbreeding depression. This should be due to numerous deleterious alleles that reduce plant fitness, when these alleles are homozygously present. Therefore, we tested the segregation of 36 markers in the six F3 populations mentioned (see Figure 5).

These markers, that were chosen because they are informative for the parent pairs used in this study, were also tested for the frequencies in 19 diploid potato genotypes, that were chosen as donors for the breeding programme (see Example 3, Figure 4 and Table 9). If lethal alleles are present, then loci with homozygous alleles should be absent. Indeed, seven markers (designated PotSNP165, PotSNP205, PotSNP753, PotSNP 238, PotSNP 700, PotSNP 100 and PotSNP 607) did not show homozygous alleles that were different from the *Sli*-donor alleles in this diploid germplasm. Most of these markers were already fixed in the F2, most of them for *Sli* donor alleles as these were more abundant in the parents, but also examples were observed whereby the F3 population was fixed for the allele of the non-*Sli* parent (PotSNP753 in populations 126B and 127). Three of these seven markers (PotSNP205, PotSNP753 and PotSNP100) segregated in the F3 populations, whereby the PotSNP753 segregated normally and PotSNP205 and PotSNP100 showed distorted segregation. These may represent deleterious alleles. Only one plant with homozygous PotSNP100 donor alleles was identified. Without wishing to be bound by theory, it is believed that this may represent a very deleterious allele. As a single plant has been identified with a homozygous allele of the donor parent, this does not provide full proof for an absolute lethal allele.

F3 x F3 crosses

The 265 F3 plants tested were raised in an unheated greenhouse in the summer of 2010. In addition about 600 additional F3 plants were raised in the field with clay soil (see also Example 2). All these plants were from unselected F2 plants grown in a winter-nursery in The Netherlands (see Example 2). Crosses were made by applying pollen from greenhouse and field grown self-compatible plants on stigma of greenhouse grown self-compatible F3 plants. Only crosses were made between F3 plants with different great-grandparents (F3 plants of populations 121 - 123 crossed with 126 – 127). The greenhouse plants designated 126-24, 126-31, 126B-2 and 127-21 were successfully crossed as female

self-compatible parent with a self-compatible F3 plant from population 123 as male parent. The female F3 plants were more than 80% homozygous, while the F3 plant in the field was not tested but the population mean homozygosity level was 94% with a range from 88 to 100%.

The parents of the F3 x F3 crosses were compared for loci that are already fixed in the F3 populations (see Table 8). Ten homozygous loci on nine chromosomes were identified that were at least contrasting between one of the four female and the one male parent used for the F3 x F3 crosses. As a consequence, the F1 hybrid progeny will be at least heterozygous for those contrasting loci. As each individual parent pair contained at least five contrasting loci, each individual progeny plant will harbour at least five contrasting chromosome regions.

Table 8. Contrasting fixed loci between the parents of the F3 x F3 crosses.

		chr	126-24	126-31	126B-2	127-21
Homozygosity level	%		86	80	88	88
Contrasting homozygous loci with F3 population 123	PotSNP392	1	x			
	PotSNP038	2	x	x		x
	PotSNP985	3			x	x
	PotSNP1105	5	x	x	x	x
	PotSNP753	6			x	
	PotSNP124	7				x
	PotSNP162	9	x	x	x	
	PotSNP021	10			x	x
	PotSNP120	10	x	x	x	x
	PotSNP213	12	x	x		
Total #	10	9	6	5	6	6

The codes 126-24, 126-31, 126B-2 and 127-21 refer to individual F3 plants that were used as females in crosses with one field grown plant of the F3 population designated "123" (see above). The homozygosity level was calculated as the percentage of homozygous loci over all loci tested (Figure 5). The "x" refers to homozygous markers that with contrasting alleles between the four female plants and the one plant of population 123.

Conclusions

It was found that after two generations of selfings homozygous diploid potato plants were obtained comprising alleles from both parents. Not a single marker out of 36 markers tested provided proof for a lethal allele. Furthermore, plants with more than 84% homozygous loci can be successfully crossed to obtain hybrid plants with at least five

heterozygous chromosome regions. This means that hybrid seeds that are essentially uniform and that represent true potato seed have now been produced.

Table 9. Allele frequencies of genome wide SNP markers in diploid germplasm and F3 populations.

SNP marker	chrom	allele frequencies											
code	no	diploid germplasm				F3 from H X D1				F3 from H x D2			
-	-	HH**	HD	DD	HD	HH	HD	DD	HD	HH	HD	DD	HD
-	-	#*	#	#	%***	#	#	#	%	#	#	#	%
PotSNP834	1	4	9	5	50	47	79	17	55	122	0	0	0
PotSNP392	1	5	11	3	58	9	32	14	58	122	0	0	0
PotSNP165	*1*	*13*	*5*	*0*	*28*	*142*	*0*	*0*	*0*	n.i.			
PotSNP038	2	2	12	5	63	15	48	80	34	122	0	0	0
PotSNP947	2	8	9	1	50	118	13	12	9	122	0	0	0
PotSNP985	3	3	8	6	47	62	17	63	12	18	46	58	38
PotSNP796	3	2	12	5	63	142	0	0	0	122	0	0	0
PotSNP960	4	6	10	3	53	59	61	20	44	n.i.			
PotSNP569	4	13	2	4	11	n.i.				121	0	0	0
PotSNP928	4	5	13	1	68	141	0	0	0	88	19	15	16
PotSNP184	5	2	7	10	37	61	59	23	41	122	0	0	0
PotSNP1105	5	2	12	5	63	140	0	0	0	19	40	62	33
PotSNP205	*6*	*13*	*6*	*0*	*32*	n.i.				*91*	*27*	*3*	*22*
PotSNP753	*6*	*9*	*10*	*0*	*53*	*28*	*20*	*91*	*14*	n.i.			
PotSNP626	6	9	8	2	42	n.i.				122	0	0	0
PotSNP081	6	12	6	1	32	142	0	0	0	n.i.			
PotSNP124	7	8	9	2	47	85	0	55	0	n.i.			
PotSNP229	7	7	11	1	58	56	0	0	0	15	37	69	31
PotSNP138	*7*	*9*	*10*	*0*	*53*	*55*	*0*	*0*	*0*	*121*	*0*	*0*	*0*
PotSNP1026	8	10	6	3	32	n.i.				80	0	42	0
PotSNP700	*8*	*9*	*9*	*0*	*50*	*140*	*0*	*0*	*0*	*34*	*0*	*0*	*0*
PotSNP116	8	7	9	3	47	140	0	0	0	n.i.			
PotSNP102	9	3	12	2	71	141	0	0	0	122	0	0	0
PotSNP458	9	11	7	1	37	n.i.				122	0	0	0
PotSNP162	9	14	4	1	21	11	31	98	22	n.i.			
PotSNP021	10	0	10	9	53	33	55	53	39	35	0	0	0
PotSNP152	10	0	8	11	42	141	0	0	0	82	23	17	19
PotSNP120	10	9	9	1	47	n.i.				48	23	51	19
PotSNP100	*11*	*14*	*5*	*0*	*26*	*53*	*89*	*1*	*62*	n.i.			
PotSNP607	*11*	*14*	*5*	*0*	*26*	n.i.				*122*	*0*	*0*	*0*
PotSNP580	11	2	5	12	26	142	0	0	0	43	53	24	44
PotSNP099	12	1	10	8	53	56	0	0	0	120	0	0	0
PotSNP996	12	7	8	4	42	142	0	0	0	n.i.			
PotSNP182	12	6	10	3	53	n.i.				56	43	21	36
PotSNP213	12	9	8	1	44	22	38	80	27	n.i.			
PotSNP052	12	4	8	7	42	142	0	0	0	89	18	14	15

#: number of plants; HH: homozygous *Sli* donor locus; DD: homozygous donor locus; HD: heterozygous locus; % is percentage heterozygous plants, low numbers indicate high level of homozygosity in F3.

Table 9. Allele frequencies of genome wide SNP markers in diploid germplasm and F3 populations.

SNP marker	chrom	allele frequencies											
code	no	diploid germplasm				F3 from H X D1				F3 from H x D2			
		HH**	HD	DD	HD	HH	HD	DD	HD	HH	HD	DD	HD
		#*	#	#	%***	#	#	#	%	#	#	#	%
PotSNP834	1	4	9	5	50	47	79	17	55	122	0	0	0
PotSNP392	1	5	11	3	58	9	32	14	58	122	0	0	0
PotSNP165	*1*	*13*	*5*	*0*	*28*	*142*	*0*	*0*	*0*	n.i.			
PotSNP038	2	2	12	5	63	15	48	80	34	122	0	0	0
PotSNP947	2	8	9	1	50	118	13	12	9	122	0	0	0
PotSNP985	3	3	8	6	47	62	17	63	12	18	46	58	38
PotSNP796	3	2	12	5	63	142	0	0	0	122	0	0	0
PotSNP960	4	6	10	3	53	59	61	20	44	n.i.			
PotSNP569	4	13	2	4	11	n.i.				121	0	0	0
PotSNP928	4	5	13	1	68	141	0	0	0	88	19	15	16
PotSNP184	5	2	7	10	37	61	59	23	41	122	0	0	0
PotSNP1105	5	2	12	5	63	140	0	0	0	19	40	62	33
PotSNP205	*6*	*13*	*6*	*0*	*32*	n.i.				*91*	*27*	*3*	*22*
PotSNP753	*6*	*9*	*10*	*0*	*53*	*28*	*20*	*91*	*14*	n.i.			
PotSNP626	6	9	8	2	42	n.i.				122	0	0	0
PotSNP081	6	12	6	1	32	142	0	0	0	n.i.			
PotSNP124	7	8	9	2	47	85	0	55	0	n.i.			
PotSNP229	7	7	11	1	58	56	0	0	0	15	37	69	31
PotSNP138	*7*	*9*	*10*	*0*	*53*	*55*	*0*	*0*	*0*	*121*	*0*	*0*	*0*
PotSNP1026	8	10	6	3	32	n.i.				80	0	42	0
PotSNP700	*8*	*9*	*9*	*0*	*50*	*140*	*0*	*0*	*0*	*34*	*0*	*0*	*0*
PotSNP116	8	7	9	3	47	140	0	0	0	n.i.			
PotSNP102	9	3	12	2	71	141	0	0	0	122	0	0	0
PotSNP458	9	11	7	1	37	n.i.				122	0	0	0
PotSNP162	9	14	4	1	21	11	31	98	22	n.i.			
PotSNP021	10	0	10	9	53	33	55	53	39	35	0	0	0
PotSNP152	10	0	8	11	42	141	0	0	0	82	23	17	19
PotSNP120	10	9	9	1	47	n.i.				48	23	51	19
PotSNP100	*11*	*14*	*5*	*0*	*26*	*53*	*89*	*1*	*62*	n.i.			
PotSNP607	*11*	*14*	*5*	*0*	*26*	n.i.				*122*	*0*	*0*	*0*
PotSNP580	11	2	5	12	26	142	0	0	0	43	53	24	44
PotSNP099	12	1	10	8	53	56	0	0	0	120	0	0	0
PotSNP996	12	7	8	4	42	142	0	0	0	n.i.			
PotSNP182	12	6	10	3	53	n.i.				56	43	21	36

PotSNP213	12	9	8	1	44	22	38	80	27	n.i.			
PotSNP052	12	4	8	7	42	142	0	0	0	89	18	14	15

#: number of plants; HH: homozygous *Sli*-donor locus; DD: homozygous donor locus; HD: heterozygous locus; % is percentage heterozygous plants, low numbers indicate high level of homozygosity in F3.

Example 5. Breeding scheme for a commercial potato variety

Starting from F3 materials obtained from crosses between an *Sli* donor and another diploid potato as described in Examples 2 and 4 above, a breeding scheme for producing commercial potato varieties is set out herein below. This scheme consists of a number of consecutive steps, which, in combination with the teaching as described herein, will allow the skilled person to arrive at a diploid or tetraploid hybrid potato variety that is as good as or preferable better then existing commercial hybrids and that can be multiplied much faster than conventional bred varieties within five years.

1. Definition of the product variety

Based on the invention as described herein, the production of a novel potato variety based on the production of commercial hybrids is not a matter of chance, but a directed design process, starting with the articulation of the product profile of the variety. Herein, the desired product profile (target product profile) for a specific market (chips potatoes, starch industry, consumption potato must be defined. In addition, evaluation methods for the relevant traits in the product profile must be defined. Preferably, the presence of relevant traits is assessed by diagnostic markers. Such markers are well known in the art of conventional potato breeding.

2. Field trials

Depending on the season at which the development process is started, a suitable trial field is selected. When starting in September or October, it is preferred that a suitable trial field located on the southern hemisphere is selected. Field trials may start with at least 2, preferably more F3 populations, selected from at least one, but preferably more crosses of a diploid *Sli* donor and another diploid potato genotype serving as a donor for an agronomical trait, preferably the trait of vigour, which F3 populations represent the diploid, fertile, self-compatible, essentially homozygous and vigorous potato lines of the invention as exemplified in Example 2. In addition to these F3 populations, F1 progenies are taken from at least two, preferably more selected F2 plants (as exemplified in Example

2 herein above) crossed with at least two, preferably more diploid potato genotypes that are different from the grandparents of the selected F2 plants. At least 50, preferably more individual plants of these F3 and F1 populations are then grown on the selected potato field and are selfed.

3. Evaluations and selection

During the growth in the field, the frequency of self-compatible plants that carry berries within a period of 2-6 weeks following the onset of flowering is evaluated, and at least two, preferably more F3 and F1 populations with at least 10%, preferably more self-compatible plants are selected. Further, during the growing season, the agronomic characteristics as defined in the product profile of the self-compatible plants in the selected populations is evaluated. Leaf samples of all self-compatible plants of the selected populations are collected for DNA marker testing.

At the end of the growing season the tuber quality and quantity of the self-compatible plants in the selected populations is evaluated, and the DNA marker genotypes is assessed by using diagnostic and random markers. Based on this evaluation and assessment, at least the five, preferably more, self-compatible plants of each population are selected based on: (i) the combination of traits that is closest to the target product profile; (ii) the highest level of homozygosity, and (iii) with the maximum number of contrasting homozygous loci. The selected and selfed F3 and F1 plants provide F4 and F2 progenies, respectively, in the form of seeds.

4. Tuber and seeds harvest and shipment

The tubers of the selected F3 and F1 plants as well as the F2 and F4 seeds in the berries of these selected plants are harvested at a period of at least at six weeks, preferably more, after flowering. The F4 seeds are collected for further field trials involving testing of the general combining abilities between F4 plants grown thereform, and the F2 seeds provide additional gene pools for breeding and are collected for further field trials. Depending on the season and in order to safe time, the seeds may suitably be shipped to the northern hemisphere for growing in a greenhouse, provided that all phytosanitary regulations of the countries involved are met.

5. Tuber storage and evaluation

The tubers of the selected F1 and F3 plants are preferably stored in the region of cultivation to avoid undesired spread of pathogens and pests, and these stored tubers are

evaluated for some relevant intrinsic tuber traits of the stored tubers as defined in the product profile.

6. Tuber propagation and evaluations after 1 year

The tubers of the selected F1 and F3 plants are grown the next season, preferably in the same region as the original potato field to avoid spread of unwanted pathogens or pest. At least two, preferably more potato tubers are used per plot and preferably one preferably more replicates per clone are used. At least one, preferably more relevant commercial cultivars are included as controls. Side-rows with commercial cultivars are preferably included to avoid side effects. The relevant plant characteristics as defined in the product profile (e.g. cooking, frying, chips making, starch content, etc) are evaluated. Further evaluated are the tuber yield per plot as well as the intrinsic relevant tuber characteristics as defined in the product profile. Such evaluations are used for family characterisation and progeny selections. These trials are repeated each year, preferably at locations in both hemispheres to continuously select the ancestor properties of plants, grown in the field. At later stages, when hybrids are obtained that are tested for commercial introductions, the evaluation trials are extended to at least two, preferably more, replicates at plot sizes of at least one, preferably more m^2 and at two, preferably more, locations preferably in but not restricted to the target markets.

7. Testcrosses with F4 seeds and field trials

Soon after the collection of the F4 progeny seeds of the selected selfed F3 plants, at least fifty, preferably more, of these seeds are sown. If needed, this sowing may occur in a heated greenhouse. The seedlings are preferably transplanted in small pots about 1 month after sowing and then transferred to the field in a suitable location some weeks thereafter. Evaluations and selections as described above are continued and at least two, preferably more selected F4 plants per population are testcrossed with at least two, preferably more, other selected F4 progeny plants of two, preferably more selected parents with maximum number of contrasting loci to produce test hybrids (F4 x F4 -> hybrid). Additionally, at least two, preferably more selected F4 plants per population may be crossed with at least one, preferably more diploid genotypes with desirable characteristics or traits that are missing in the selected plants to enhance the genetic diversity of the breeding germplasm. Further, at least two, preferably more selected F4 plants per population may be selfed to produce F5 seeds. Still further, plant parts of at least two, preferably more selected F4 plants per population may be sterilised, *in vitro* propagated and treated with suitable chemicals (colchicine / oryzaline) to produce tetraploid F4

genotypes, and testcrosses are made with tetraploid F4 plants between the same parents as described above. In addition, similarly F2 progeny seeds are sown, transplanted and evaluated, crossed and selfed but not test/crossed

The above breeding scheme is repeated each year whereby an incremental increase in plant traits as defined in the product profile is achieved. The evaluations of the tubers from testcrosses are instrumental to determine the general combining abilities of parents. General combining ability is defined herein as the average performance of a line in a series of crosses. For each market at least two, preferably more elite parent lines are achieved that will render commercial hybrid seeds. It is expected that in five years a diploid or tetraploid hybrid variety is generated that is as good as or preferable better then existing commercial hybrids and that can be multiplied much faster than conventional bred varieties.

UNMARKED
SUBSTITUTE SPECIFICATION

Title: Hybrid Seed Potato Breeding

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of United States Application No. 13/504,156, filed July 10, 2012 (pending), which application is a 371 National Stage application of PCT/NL10/50716 which application claims the benefit of priority to United States Provisional Application No. 61/254,980, filed October 26, 2009, and to European Patent Application No. 09013474.3, filed on October 26, 2009, each of which are herein incorporated by reference in their entireties.

FIELD OF THE INVENTION

The invention relates to the field of agriculture, in particular to new plants and processes for obtaining them. The invention also relates to methods for improving the genetic constitution of crop plants, and to plants thus obtained. The invention further relates to a method for producing hybrid seeds and to hybrid seeds thus produced and to methods of producing crops using the hybrid seeds of the invention.

BACKGROUND OF THE INVENTION

The potato (*Solanum tuberosum* L.) is an integral part of the global food system. It is the world's number one non-grain food commodity, with production reaching a record 325 million tonnes in 2007.

Unlike other major field crops, potatoes are reproduced vegetatively from other potatoes. Therefore, a part of each year's crop - ranging from 5 to 15 percent, depending on the quality of the harvested tubers - is set aside for re-use in the next planting season. Most farmers in developing countries select and store their own seed tubers. In developed countries, farmers are more likely to purchase disease-free "certified seed" from dedicated suppliers.

Seed potatoes are more difficult to produce and supply than grain or pulse seed. A seed:harvest ratio of 1:20 for potatoes is considered good, compared to 1:400 for maize or 1:10.000 for tomato. One hectare may therefore require two tonnes of seed material to maximize the yield of harvestable products, compared to 18 kg for maize. In order to break dormancy, seed potatoes should be stored for several weeks before they can be planted. The right conditions during storage such as amount of light, temperature and humidity are crucial to ensure good "seed" quality.

In addition to the poor seed:harvest ratio, seed potatoes attract and transport pests and diseases. These include (amongst others) late blight, Andean potato weevils, nematodes, tuber moths and viruses. The latter are transmitted in the field by aphids and then carried from generation to generation in the seed. Such a virus infection can decrease yields by up to 20 percent.

Seed potatoes have high transportation costs because of the great distances between the major seed production areas and the major consumer production areas and the relatively high weight of individual seed potato tubers.

To feed the growing world population now and in future, the potato industry has to keep growing to meet the needs of the consuming public. Substantial research and development efforts are devoted to the modernization of planting and harvesting of fields and processing of potatoes, and to the development of economically advantageous potato varieties. Through crossbreeding of potatoes, researchers hope to obtain potatoes with the desirable characteristics of good processing, both for fresh consumption as well as for industrial purposes, high soluble solids content, high yield, resistance to diseases and pests and adaptability to various growing areas and conditions.

The research leading to potato varieties which combine the advantageous characteristics referred to above is largely empirical. This research requires large investments of time, manpower, and money. The development of a potato cultivar can often take up to eight years or more followed by at least five years of propagation to get sufficient quantities for commercial usage. Breeding begins with careful selection of superior parents to incorporate the most important characteristics into the progeny. Since all desired traits usually do not appear in one progeny, breeding is a continuous process of selecting the best recombinants, combining favourable traits of the ancestors.

The arduous task of producing a new potato variety is best understood when understanding the genetics of potato. The commercial potato has a tetraploid genome. Diploid tubers are generally too small for important commercial applications. In addition, the tetraploid genome is extremely heterozygous, often harbouring multiple alleles per locus. It is believed that self-incompatibility, which is primarily experienced at diploid level, and inbreeding depression are responsible for the maintenance of the high genetic variability found in potato and that overdominance of heterozygous alleles (heterosis) results in vigorous plants. In a typical potato offspring obtained from a cross between two unrelated parental lines deleterious alleles may therefore contribute to either a reduced fitness in case of homozygosity or an increased vigour in case of heterozygosity. It is clear that a breeder needs large populations to maximise the chance of finding individuals that

carry a relatively high number of heterozygotic loci and a low number of homozygotic loci, while also exhibiting beneficial combinations of agronomically desirable traits.

Present potato breeding techniques rely on the controlled crossing of parental clones which themselves are the result of a comprehensive pre-breeding development during which amongst others special techniques such as chromosome doubling, embryo rescue, and somatic fusion are applied in order to introduce the beneficial characteristics of for instance wild and primitive *Solanum* species into these clones. The parental material that is found suitable for further breeding after a phenotypic selection procedure is then mutually crossed and the resulting non-uniform hybrid seeds are sown in large numbers in greenhouses. From tens of thousands of individual F1 seedlings tubers are harvested and retained for the next year's planting. The next year a single "seed" tuber from each resulting seedling is planted in the field. Extreme caution must be taken to avoid the introduction of viruses and diseases since the material is only clonally (vegetatively) expanded before it is sold to individual customers years later. After the second year, samples of tubers are taken for density measurements and preliminary fry tests to determine the suitability of the tubers for commercial use. A multitude of tubers of plants which have survived the selection process to this point are then planted in the third year for a more comprehensive series of fry tests and density determinations. At the fourth-year stage of development, a diminishing number of surviving selections is grown in ever expanding numbers and plants thereof are subjected to field trials in several stages to determine their adaptability to different growing conditions. Eventually, the varieties having superior agronomical qualities are transferred to other farms and the "seed" (in the form of tubers) is increased to commercial scale. Since one "seed" tuber may generate between 6 and 20 harvested tubers this up-scaling process may take years before sufficient "seed" is produced. Generally, by this time, eight or more years of planting, harvesting and testing have been invested in attempting to develop a new and improved potato cultivar.

To reduce inbreeding depression a breeder may introduce new genes from a genetically more remote parent such as from wild and primitive species with ploidy levels ranging from diploid to hexaploid. However, when two genetically unrelated potato plants are crossed, the level of heterozygosity may be increased but simultaneously more deleterious genes are also introduced. As a consequence, a breeder will typically make additional crosses with more commercial germplasm to enrich the population for favourable alleles. All together such a multiple crossing breeding programme may take dozens of years as the selection of the favourable genotypes in each generation may already take five years. Therefore, potato breeding is currently a predominantly empirical exercise, strongly characterised by trial and error.

Potatoes and their related wild species (tuber-bearing *Solanum* species) are mostly outbreeding because self fertilisation is hampered by a gametophytic self-incompatibility system. Self-incompatibility (SI) is a general name for several genetic mechanisms in angiosperms, which prevent self-fertilization and inbreeding. In plants with SI, when a pollen grain produced in a plant reaches a stigma of the same plant or another plant with a similar genotype, the process of pollen germination, pollen tube growth, ovule fertilization, and embryo development is halted at one of its stages, and consequently no seeds are produced. Self-incompatibility is not found in tetraploid potatoes.

The provision of such self-compatible clones may facilitate the generation of selfed progenies of potato, and hence the production of (highly) homozygous potato lines. This could provide a great opportunity to the development of homozygous elite breeding lines in potato. However, to date, the development of homozygous elite lines with genetically fixed agronomically desirable traits, that would enable the production of genetically uniform hybrid potato seed, has been unsuccessful.

The provision of homozygous elite lines is hampered by unknown causes. Selfing of an occasionally encountered self-compatible clone results in a slower decrease of heterozygosity than theoretically expected. The slower decline of heterozygosity may be the result of unintentional but unavoidable selection during selfing for the heterozygous plants in the offspring that exhibit higher vigour, fertility and seed germination. It is implied that, as the heterozygosity is reduced by selfing, the fertility and vigour are also reduced and the plants may become weak and completely sterile. The situation is perhaps worsened by the establishment of homozygous configurations of recessive deleterious genes. This phenomenon which is generally referred to as inbreeding depression has greatly hampered the development for homozygous potato lines and hence the production of uniform hybrid potato seed.

There is a large prejudice against the production of homozygous breeding lines in potato due to inbreeding depression. Uijtewaal et al. (Euphytica 36 (1987) 745-753) indicated that owing to sterility problems, homozygous potato clones would be of little importance for practical breeding. Developing homozygous inbred lines was considered, but rendered impossible in potato (Umaerus, 1987, Proceeding of the 10th Triennial Conference of the European Association of Potato Research, Aalborg, Denmark, pp 72–103 as cited in Almekinders et al. 2009 Potato Research 52:275–293). Routes involving doubling of haploids have long been presumed as promising. Nontheless, up to the present day the ruling opinion is that inbreeding depression in diploid potato is too strong to ever result in vigorous homozygous plants.

Birhman and Hosaka (Genome 43: 495–502 (2000)) have suggested the possibility to utilize the *Sli* gene derived from *S. chacoense* for development of highly homozygous true potato seed (TPS) lines and heterosis breeding of the potato. However, to date, no homozygous lines with good agronomic traits such as good tuber yield, have ever been reported from this proposed line of research. Rather, those homozygotes that have been produced do not exhibit any agronomically relevant tuber yield.

Rommens in 2010 (Genetic modification of Plants, Kempen & Jung , eds, In: Biotechnology in Agriculture and Forestry 64(1): 61-77 (2010)), advocates the route of genetic transformation, due to the fact that efforts to improve the yield and quality of this crop are hampered by inbreeding depression.

In short, production of true breeding lines of potato is considered impossible. Due to this fact, potato breeding cannot escape the traditional schemes based on crossing of tetraploid heterozygotes. As a result, the challenge is considered formidable to combine various utilization traits (relating to fresh and processing uses), resistances to pathogens and pests, and numerous other relevant agronomic traits with improvements in yield into a commercially acceptable cultivar (Douches et al. 1999, Crop Science 36(6):1544-1552).

It is an objective of the present invention to provide means and methods for the production of elite breeding lines of potato and for the production of uniform hybrid potato seed from which plants can be grown that exhibit agronomically relevant tuber yield.

SUMMARY OF THE INVENTION

The present inventors have attempted to achieve homozygous parental breeding lines for generative propagation of potatoes via a number of routes, none of which were successful. These unsuccessful routes included androgenesis, gynogenesis, doubling of haploids and continued selfings. Only by repeated selfing of a self-compatible plant comprising the *Sli* gene derived from *S. chacoense* and the use of did the inventors arrive at the product of the present invention. The present invention now provides in a first aspect a diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant. Plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant are in general terms also referred to herein as being vigorous.

In a preferred embodiment, said line in addition to said tuber yield comprises at least one, more preferably at least 2, 3, 4, 5 or 6 (additional) agronomically desirable traits. Preferably, said agronomically desirable traits are selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to

resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, and cold sweetening resistance.

In another preferred embodiment of said potato line of the invention, plants of said line when mature attain an average fresh weight of the foliage and shoots of at least 500 grams per plant. Plants attaining an average fresh weight of the foliage and shoots of at least 500 grams per plant are in general terms also referred to herein as being vigorous.

In another aspect, the present invention provides a plant of a line according to the invention as described above. Preferably, said plant is diploid, fertile, self-compatible and essentially homozygous and has an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant.

In another aspect, the present invention provides a seed of a plant as described above. Said seed may be homozygous (i.e. represent a selfed seed), but may also be heterozygous for the alleles where the parents differ. In one embodiment, said seed is preferably a uniform hybrid potato seed. Such uniform hybrid potato seed being the result of a cross between two homozygous, self-compatible, fertile and vigorous plants of a line according to the invention as described above, preferably of two different lines, wherein the lines contain at least 20 % contrasting homozygous loci as determinable by molecular marker analysis.

In another aspect, the present invention provides a method for producing a diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, comprising:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, self-compatible, fertile and essentially homozygous potato line;

(b) providing a second potato plant, wherein said second potato plant is a plant of a diploid or tetraploid potato line which may have any level of homozygosity, said line of said second potato plant comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant;

(c) cross pollinating said first and second potato plant to provide seeds and collecting said seeds to thereby provide an offspring generation in the form of a hybrid offspring seed;

(d) growing said hybrid offspring seed into a population of hybrid offspring potato plant and selecting from said population plants producing tubers at a yield expressed in grams of fresh weight of at least 200 grams per plant, and

(e) selfing said hybrid offspring potato plant or backcrossing said hybrid offspring potato plant to a potato plant of said line of said first potato plant, for between 1 to 8 selfings or backcrosses, to thereby provide a diploid, self-compatible and essentially homozygous potato breeding line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant.

A first potato plant in a method as described herein is preferably a plant of a first diploid, self-compatible, fertile and essentially homozygous potato line, wherein the self-compatibility is conditioned by the presence of a dominant allele of an S-locus (self-incompatibility locus) inhibitor gene (Sli) that is nonallelic to the S locus as described in Hosaka and Hanneman 1998 Euphytica, 99: 191–197.

The above method, when performed on a sufficiently large scale (at least more than 10.000 plants) and performed with the use of a suitable second potato plant, which is preferably a diploid potato clone that is essentially not homozygous, said line of said second potato plant comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, preferably a line such as IVPAA-096-18 (D2) as described in the Examples herein below, results in the production of an essentially homozygous, fertile, vigorous and self-compatible breeding line, wherein said vigour is in particular represented by an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant. The provision of such a breeding line is a crucial step in the provision of genetically homozygous true potato seed (TPS).

The first and second breeding line can subsequently be used in the production of the TPS when both the first and second breeding line comprise agronomically desirable characteristics, have good general combining abilities, and preferably have at least 20% contrasting homozygous loci as determinable by molecular marker analysis.

In a preferred embodiment of said method for producing a diploid, fertile, self-compatible and essentially homozygous potato line according to the invention, said steps a-e are repeated wherein step b comprises the provision of third (or further) potato plant wherein said third potato plant is a plant of a diploid or tetraploid potato line which may have any level of homozygosity, wherein said third potato plant is a donor for an additional agronomically desirable trait. Preferably, said trait is selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, cold tolerance, drought tolerance, wet

tolerance, tolerance to dry and wet rot, salinity tolerance, and cold sweetening resistance, or any other agronomically beneficial trait or desirable characteristic as defined herein.

The second or third potato plant as described above may suitably be a diploid potato clone selected from the plants D1-D21 as listed in Table 6 of Example 3 below. The skilled person will understand that other diploid potato clones may also be used. Highly preferred is the use of potato clone IVPAA-096-18 (D2) which is available from Laboratory of Plant Breeding (IVP) (Dr. Ir Ronald B.C. Hutten), Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.

Proper (commercial) sources of tetraploid cultivars are provided through the European Cultivated Potato Database (ECPD) at http://www.europotato.org/menu.php and/or the Potato Pedigree Database of the expertise group Plant Breeding (a cooperation between the Laboratory of Plant breeding of Wageningen UR, the Netherlands) and the business unit Biodiversity and breeding of Plant Research International (PRI), Wageningen, the Netherlands at http://www.plantbreeding.wur.nl/potatopedigree/.

In another preferred embodiment of said method, said first potato plant is a plant of potato line NCIMB 41663, NCIMB 41664, NCIMB 41665, or NCIMB 41765, representative seeds of said line having been deposited with the NCIMB, Aberdeen, Scotland.

In yet another preferred embodiment of said method, said second potato plant is a plant of a second diploid, fertile, self-compatible and essentially homozygous potato line.

In yet a further preferred embodiment of said method, said second potato plant is a plant of a second diploid, fertile, self-incompatible potato line.

The method for producing a diploid potato breeding line may further comprise the steps of enhancing the breeding value of the diploid potato breeding line by intercrossing, selfing and backcrossing while continuous selecting for agronomically desirable traits. Experimental crosses are done to evaluate general combining abilities (intercrossability).

In another aspect, the present invention provides a diploid potato breeding line, more preferably an elite line, obtainable by the method of the present invention as described above.

In another aspect, the present invention provides a hybrid potato seed obtainable by crossing plants of a first and second diploid potato breeding line according to the present invention as described above, wherein said plants of said first and second

diploid potato breeding line contain at least 20 % contrasting homozygous loci as determinable by molecular marker analysis.

In another aspect, the present invention provides a method for producing a uniform hybrid potato seed, comprising:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, fertile, self-compatible and essentially homozygous potato line,

(b) providing a second potato plant, wherein said second potato plant is a plant of a second diploid, fertile, self-compatible and essentially homozygous potato line, wherein said first and second plant contain at least 20 % contrasting homozygous loci as determinable by molecular marker analysis, and

(c) cross pollinating said first and second potato plant and allowing one of said parent plants to produce berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed.

In a preferred embodiment of a method for producing hybrid seed, said first and second potato plant are from potato lines comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, preferably wherein said first and second potato plant are plants having a tuber yield of at least 200 grams fresh weight per plant.

In another preferred embodiment of said method of producing hybrid seed, at least one of said first and second potato plants comprises at least one agronomically desirable trait, preferably selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, and cold sweetening resistance.

In another preferred embodiment of a method for producing hybrid seed according to the present invention, said first potato line is a diploid, self-compatible and essentially homozygous offspring potato plant comprising the *Sli*-gene, preferably wherein said first potato plant is a plant of potato lines NCIMB 41663, NCIMB 41664, NCIMB 41665, or NCIMB 41765, representative seeds of said lines having been deposited with the NCIMB, Aberdeen, Scotland, or a diploid, self-compatible and essentially homozygous offspring potato plant thereof.

In another preferred embodiment of a method for producing hybrid seed according to the present invention, said second potato plant is a plant of a second diploid, self-compatible and essentially homozygous potato line preferably comprising at least two

agronomically desirable traits selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, and cold sweetening resistance.

In another aspect, the present invention provides a seed of potato lines NCIMB 41663, NCIMB 41664, NCIMB 41665, or NCIMB 41765, representative seeds of said line having been deposited with the NCIMB, Aberdeen, Scotland.

In another aspect, the present invention provides a seed produced by the method for producing a uniform hybrid potato seed as disclosed herein.

In another aspect, the present invention provides a hybrid potato plant, or part thereof, produced by growing the seed of the present invention.

In another aspect, the present invention provides pollen or an ovule of the hybrid potato plant, or part thereof, of the present invention.

In another aspect, the present invention provides a tuber from the hybrid potato plant of the present invention.

In another aspect, the present invention provides a potato plant having all of the physiological and morphological characteristics of the hybrid potato plant produced by growing the seed of the present invention.

In another aspect, the present invention provides a tissue culture of the hybrid potato plants of the present invention.

In another aspect, the present invention provides a potato plant regenerated from the tissue culture of the present invention, wherein said regenerated potato plant has all the physiological and morphological characteristics of the hybrid potato plant produced by growing the seed of the present invention.

In another aspect, the present invention provides a method for producing a hybrid potato seed wherein the method comprises crossing a first potato plant with a

second potato plant and harvesting the resultant hybrid potato seed, wherein said first and/or second parent potato plant is a diploid, fertile, self-compatible and essentially homozygous plant according to the present invention that has an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant.

In another aspect, the present invention provides a method of producing a hybrid potato plant, said method comprising producing a seed according to a method of the present invention and growing said seed into a potato plant.

In another aspect, the present invention provides a potato plant produced by the method of the invention, or a tuber thereof.

In another aspect, the present invention provides a method set of at least 4 intercrossable homozygous diploid, fertile, self-compatible and essentially homozygous potato lines comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant according to the present invention, wherein each of said lines differ from another line in said set by having at least 20 % contrasting homozygous loci as determinable by molecular genetic marker analysis.

The invention also provides as an embodiment of methods of the invention as described above methods comprising the steps of:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, self compatible and essentially homozygous potato line carrying chromosomes with alleles for agronomically desirable traits that are to be expressed in an offspring generations,

(b) providing a second potato plant, wherein said second potato plant is a plant of a second diploid, self compatible and essentially homozygous potato line carrying chromosomes with alleles for agronomically desirable traits that are to be expressed in an offspring generations and that may be different form the traits in the said first plant, and

(c) cross pollinating said first and second potato plant such that one of said parent plants produces berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed carrying combinations of chromosomes with said alleles.

Thus, in an alternative embodiment the plant of the first potato line may carry chromosomes with alleles for agronomically desirable traits that are to be expressed in an

offspring generations, in which case the agronomically desirable trait in the plant of the second potato line may be different from the traits in the said first plant.

In another aspect, the present invention provides a method of producing a herbicide resistant, nematode resistant, insect resistant and/or disease resistant potato plant, said method comprising producing a seed according to a method of the invention described above by crossing parental lines that are herbicide resistant, nematode resistant, insect resistant and/or disease resistant and growing said seed a potato plant.

In another aspect, the present invention provides a herbicide resistant, nematode resistant, insect resistant and/or disease resistant potato plant produced by the method of the invention as described above.

In yet another aspect, the present invention provides a method for preventing transmittal of virus infections between potato generations, comprising:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, self-compatible and essentially homozygous potato line,

(b) providing a second potato plant, wherein said second potato plant is a plant of a potato line that may have any level of ploidy and/or any level of homozygosity, said second potato plant carrying a chromosome with an allele for a agronomically desirable trait that is to be expressed in an offspring generation;

(c) cross pollinating said first and second potato plant such that one of said parent plants produces berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed carrying a chromosome with said allele;

(d) growing said hybrid offspring seed into a hybrid offspring potato plant carrying a chromosome with said allele, and

(e1) harvesting tubers from said hybrid offspring potato plant to provide tubers expressing said trait, or

(e2) selfing said hybrid offspring potato plant or backcrossing said hybrid offspring potato plant to a potato plant of said line of said first potato plant, thereby providing further offspring plants carrying a chromosome with said allele and wherein said offspring plants are increasingly homozygous following each backcrossing or selfing step.

In another aspect, the present invention provides a diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, said plant being obtainable by:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, fertile, self-compatible and essentially homozygous potato line,

(b) providing a second potato plant, wherein said second potato plant is a plant of a diploid potato line having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant,

(c) cross pollinating said first and second potato plant and allowing one of said parent plants to produce berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed,

(d) growing said hybrid offspring seed into a hybrid offspring potato plant,

(e) backcrossing said hybrid offspring potato plant to a potato plant of said line of said first potato plant, and selecting from the offspring plants thus generated a plant that is diploid, self-compatible and vigorous in that it produces tubers at a yield expressed in grams of fresh weight of at least 200 grams per plant, and

(f) selfing said diploid, self-compatible and vigorous plant for between 1 to 8 generations while selecting for self-compatibility and vigour as defined in (e).

In another aspect, the present invention provides a hybrid potato seed which seed, when sown produces a diploid, fertile, self-compatible and essentially heterozygous potato plant having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, said plant being obtainable by:

(a) providing a first diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant,

(b) providing a second diploid, fertile, self-compatible and essentially homozygous potato line comprising plants having an average tuber yield expressed in grams of fresh weight of at least 200 grams per plant, wherein said first and second plant contain at least 20 % contrasting homozygous loci as determinable by molecular marker analysis, and

(c) cross pollinating said first and second potato plant and allowing at least one of said parent plants to produce berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed.

The steps of selecting or providing plants that are homozygous, or the step of selecting or providing plants having at least 20% contrasting loci as defined herein above, may comprise marker assisted selection using the molecular genetic markers as provided herein.

DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a schematic presentation of a development scheme for the development of hybrid potato cultivars using the methods and plants of the present invention, wherein the various steps in obtaining commercial potato hybrids are indicated:

Box 0, Potato germplasm: The origin comprises in principle all potato genotypes within the cultivated potato species (*Solanum tuberosum*) and related crossable species;

Box 1, Self-compatible diploid potato: Cultivated potato (*Solanum tuberosum*) is usually self incompatible. Self-compatibility is introduced from related species (see for instance Hermsen, J.G.Th. 1978, Euphytica 27, 1-11);

Box 2, Homozygous fertile self-compatible diploid potato: Homozygous diploids can be obtained by doubling haploids (Uijtewaal et al., 1987, Theor. Appl. Genet. 73, 751-78) by anther culture (Jacobsen and Sopory, 1977, Theor. Appl. Genet. 52, 119-123), by ovule culture or by repeated selfings (Phumichai & Hosaka, 2006, Euphytica 149, 251-258). The example of the latter is illustrated herein;

Box 3, Vigorous fertile homozygous self-compatible diploid potato: The vigour is enhanced by crossing with other diploid potato, selfing and backcrossing while selecting for higher vigour;

Box 4, Diploid potato breeding Lines: Agronomically desirable traits are introduced by crossing with other diploid potato, selfing and backcrossing while selecting for these traits;

Box 5, Elite diploid potato breeding Lines: The breeding value is further enhanced by intercrossing, selfing and backcrossing while continuous selecting for agronomically desirable traits. Experimental crosses are done to evaluate general combining abilities;

Box 6 Potato hybrid cultivars: Good combining breeding lines are crossed. The seeds obtained are hybrid true potato seeds that are uniform.

FIG. 2 shows a schematic presentation of the pedigrees of the populations, deposits and plants as described in the Examples, starting from a cross between a diploid, essentially homozygous and self-compatible potato plant ("H") and three different diploid clones (D1, D2, and D3) that are essentially non-homozygous and self-incompatible and that act as donor lines of one or more desirable agronomic traits. The production of an array of essentially homozygous lines from backcross lines to fully selfed clones, each having one or more desirable traits derived from the donor lines provides a set of elite breeding lines for the production of an array of hybrid potato cultivars in the form of hybrid seed. ⊗ Refers to selfings. As can be seen, and as detailed in the Examples, only

donor line D2 resulted in the production of a large number of self-compatible plants that would show vigour as defined herein. Vigorous plants could be obtained from the other crossings as well, albeit at a much lower frequency (0.1-1%).

FIG. 3A - FIG. 3F display the ultra-dense genetic linkage map of the genome of diploid breeding clone RH89-039-16. The number on the left of the linkage group indicates the cumulative number of recombination events counted from the top. The number of AFLP markers in each bin is represented by shades of grey according to the figure legend. Putative centromere positions are indicated with "Cen" alongside the chromosome (van Os et al., 2006 Genetics 173(2):1075-1087). The numbers on the right of the linkage group indicate the approximate location of the corresponding PotSNPmarker as used herein, wherein reference is made to Table 5B in Example 3, below, for marker annotations. The figure clearly indicates the distribution of informative markers over the potato genome of this clone. A similar distribution was observed for these markers with another clone used herein, clone SH83-92-488.

FIG.4A - FIG. 4L show marker data of 100 markers over 20 different diploid potato genotypes, representing the diploid potato breeding germplasm (Example 2).

FIG. 5A-1 - FIG. 5F - 4 show the result of SNP marker analysis as described in Example 3 on a set of six F3 populations and their respective parents.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

The term "potato" is used herein to refer to material that is essentially of species *Solanum tuberosum*, but may include introgression segments of other tuber bearing *Solanum* species such as *Solanum chacoense, Solanum phureja, Solanum andigena, Solanum demissum.*

The term "potato plant" is used herein to refer to a seedling or mature plant as grown from cell culture or seed. Persons of ordinary skill in the art will recognize that when the term "potato plant" is used in the context of the present invention, this also includes derivative varieties that retain the essential distinguishing characteristics of for instance potato varieties that are subject of the present invention, such as a Single Gene Converted plant of that variety or a transgenic derivative having one or more value-added genes incorporated therein (such as herbicide or pest resistance). Backcrossing methods can be used with the present invention to improve or introduce a characteristic into the variety.

The term "crossing" as used herein refers to the fertilization of female plants (or gametes) by male plants (or gametes). The term "gamete" refers to the haploid

reproductive cell (egg or sperm) produced in plants by mitosis from a gametophyte and involved in sexual reproduction, during which two gametes of opposite sex fuse to form a diploid zygote. The term generally includes reference to a pollen (including the sperm cell) and an ovule (including the ovum). "Crossing" therefore generally refers to the fertilization of ovules of one individual with pollen from another individual, whereas "selfing" refers to the fertilization of ovules of an individual with pollen from the same individual. Crossing is widely used in plant breeding and results in a mix of genomic information between the two plants crossed one chromosome from the mother and one chromosome from the father. This will result in a new combination of genetically inherited traits. Usually, the progeny of a crossing is designated as: “F1”. If the F1 is not uniform (segregates) it is usually designated as “F1 population”. “Selfing” of a homozygous plant will usually result in a genetic identical plant since there is no genetic variation. “Selfing” of an F1 will result in an offspring that segregates for all traits that have heterozygotic loci in the F1. Such offspring is designated: “F2” or “F2 population”

When referring to “crossing” in the context of achieving the introgression of a genomic region or segment, the skilled person will understand that in order to achieve the introgression of only a part of a chromosome of one plant into the chromosome of another plant, it is required that random portions of the genomes of both parental lines will be recombined during the cross due to the occurrence of crossing-over events in the production of the gametes in the parent lines. Therefore, the genomes of both parents must be combined in a single cell by a cross, where after the production of gametes from said cell and their fusion in fertilization will result in an introgression event.

The term “intercrossable”, as used herein, refers to the ability to yield progeny plants after making crosses between parent plants.

As used herein, the terms "introgressing", "introgress" and "introgressed" refer to both a natural and artificial process whereby individual genes or entire chromosomes are moved from one individual, species, variety or cultivar into the genome of another individual, species, variety or cultivar, by crossing those individuals, species, varieties or cultivars. In plant breeding, the process usually involves selfing or backcrossing to the recurrent parent to provide for an increasingly homozygous plant having essentially the characteristics of the recurrent parent in addition to the introgressed gene or trait.

The term “introgression” refers to the result of an introgression event.

The term ”backcross” refers to the result of a “backcrossing” process wherein the plant resulting from a cross between two parental lines is (repeatedly) crossed with one of its parental lines, wherein the parental line used in the backcross is referred to as the recurrent parent. Repeated backcrossing results in replacement of genome fragments of the

donor parent with those of the recurrent. The offspring of a backcross is designated "BCx" or "BCx population", where "x" stands for the number of backcrosses.

The term "backcrossing" as used herein refers to the repeated crossing of a hybrid progeny back to the recurrent parents. The parental potato plant which contributes the gene for the desired characteristic is termed the nonrecurrent or donor parent. This terminology refers to the fact that the nonrecurrent parent is used one time in the backcross protocol and therefore does not recur. The parental potato plant to which the gene or genes from the nonrecurrent parent are transferred is known as the recurrent parent as it is used for several rounds in the backcrossing protocol. In a typical backcross protocol, the original variety of interest (recurrent parent) is crossed to a second variety (nonrecurrent parent) that carries the single gene of interest to be transferred. The resulting progeny from this cross are then crossed again to the recurrent parent and the process is repeated until a potato plant is obtained wherein essentially all of the desired morphological and physiological characteristics of the recurrent parent are recovered in the converted plant, in addition to the single gene or a limited number of genes transferred from the nonrecurrent parent.

The selection of a suitable recurrent parent is an important step for a successful backcrossing procedure. The goal of a backcross protocol is to alter or substitute a single trait or characteristic in the original variety. To accomplish this, a single gene of the recurrent variety is modified, substituted or supplemented with the desired gene from the nonrecurrent parent, while retaining essentially all of the rest of the desired genes, and therefore the desired physiological and morphological constitution of the original variety. The choice of the particular nonrecurrent parent will depend on the purpose of the backcross. One of the major purposes is to add some commercially desirable, agronomically important traits to the recurrent parent. The exact backcrossing protocol will depend on the characteristic or trait being altered or added to determine an appropriate testing protocol. Although backcrossing methods are simplified when the characteristic being transferred is a dominant allele, a recessive allele may also be transferred. In this instance, it may be necessary to introduce a test of the progeny to determine if the desired characteristic has been successfully transferred. Preferably, such genes are monitored by diagnostic molecular markers.

Likewise, transgenes can be introduced into the plant using any of a variety of established recombinant methods well-known to persons skilled in the art.

Many single gene traits have been identified that are not regularly selected for in the development of a new variety but that can be improved by backcrossing and genetic engineering techniques. Single gene traits may or may not be transgenic, examples of these

traits include but are not limited to: herbicide resistance; resistance to bacterial, fungal or viral disease; insect resistance; uniformity or increase in concentration of starch and other carbohydrates; enhanced nutritional quality; decrease in tendency of tuber to bruise; and decrease in the rate of starch conversion to sugars.

The term "selfing" refers to the process of self-fertilization wherein an individual is pollinated or fertilized with its own pollen. Repeated selfing eventually results in homozygous offspring.

A "line", as used herein, refers to a population of plants derived from a single cross, backcross or selfing. The individual offspring plants are not necessarily identical to one another. It is possible that individual offspring plants are not vigorous, fertile or self compatible due to natural variability. However, it is foreseen that suitable plants that are vigorous, fertile and self compatible can be easily identified in a line and used for additional breeding purpose.

As used herein, the term "allele(s)" means any of one or more alternative forms of a gene, all of which alleles relate to at least one trait or characteristic. In a diploid cell or organism, the two copies of a gene occupy corresponding loci on a pair of homologous chromosomes. Each copy may be a distinct allele.

A "gene" is defined herein as a hereditary unit (often indicated by a sequence of DNA) that occupies a specific location on a chromosome and that contains the genetic instruction for a contribution to potential phenotypic characteristics or trait in a plant.

A "locus" is defined herein as the position that a given gene occupies on a chromosome of a given plant species.

As used herein, the term "homozygous" means a genetic condition existing when identical alleles reside at corresponding loci on homologous chromosomes.

The term "essentially homozygous" refers to a level of homozygosity of at least 25%, preferably at least 50%, more preferably at least 75%, still more preferably at least 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% homozygosity when testing 100, preferably 1000, more preferably at least 10.000 loci.

Homozygosity levels are average values for the population, and refer preferably to those loci wherein the parents differ.

As used herein, the term "heterozygous" means a genetic condition existing when different alleles reside at corresponding loci on homologous chromosomes.

The term "recombination" or "recombine" refers to the exchange of information between two homologous chromosomes during meiosis. In a "recombinant" plant, DNA that is originally present on a specific location within the chromosome, e.g.

linked to a gene/locus, is exchanged for DNA from another plant (i.e. maternal for paternal or *vice versa*). In order to exchange only the required material, and maintain the valuable original information on the chromosome as much as possible, two flanking crossover or recombination events will usually be required. In a double recombinant this exchange has taken place on both sides of a gene/locus. one way to find such a double recombinant, is to screen a population of F2-plants. This population has to be large, since double recombination occurs in only a limited frequency. Alternatively, double recombinants within a genetic unit can be the result of subsequent backcrossing. The frequency of double recombination is the product of the frequencies of the single recombinants. (E.g. a recombinant in a 10 cM area can be found with a frequency of 10%, double recombinants are found with a frequency of 10% x 10% = 1 %).

As used herein, the term "progeny" means (a) genetic descendant(s) or offspring.

As used herein, the term "population" means a genetically heterogeneous collection of plants sharing a common genetic derivation.

A "recombination event" refers to a mitotic or meiotic crossing-over event, including a GMO event.

As used herein, the term "hybrid" means any offspring of a cross between two genetically unlike individuals, more preferably the term refers to the cross between two (elite or inbred) breeding lines which will not reproduce true to the parent from seed.

The term “segregate”, as used herein, refers to the separation of paired alleles during meiosis so that members of each pair of alleles appear in different gametes. The term includes reference to the result of this genetic phenomenon wherein the offspring population of a crossing in which at least one of the parents is heterozygous for an allelic gene is non-uniform with respect to phenotypic trait conferred by said gene.

The term “breeding line”, as used herein, refers to a line of a cultivated potato having commercially valuable or agronomically desirable characteristics, as opposed to wild varieties or landraces. The term includes reference to an elite breeding line or elite line, which represents an essentially homozygous, usually inbred, line of plants used to produce commercial F_1 hybrids. An elite breeding line is obtained by breeding and selection for superior agronomic performance comprising a multitude of agronomically desirable traits. An elite plant is any plant from an elite line. Superior agronomic performance refers to a desired combination of agronomically desirable traits as defined herein, wherein it is desirable that the majority, preferably all of the agronomically desirable traits are improved in the elite breeding line as compared to a non-elite breeding line. Elite breeding lines are essentially homozygous and are preferably inbred lines.

The term "elite line", as used herein, refers to any line that has resulted from breeding and selection for superior agronomic performance. An elite line preferably is a line that has multiple, preferably at least 3, 4 5, 6 or more (genes for) desirable agronomic traits as defined herein.

The terms "cultivar" and “variety” are used interchangeable herein and denote a plant with has deliberately been developed by breeding, cq crossing and selection, for the purpose of being commercialized, cq used by farmers and growers, to produce agricultural products for own sonsumption or for commercialization (fresh consumption, processing, feed, etc). The term “breeding germplasm” denotes a plnat having a biological status other than a "wild" status, which "wild" status indicates the original non-cultivated, or natural state of a plant or accession.

The term "breeding germplasm" includes, but is not limited to, semi-natural, semi-wild, weedy, traditional cultivar, landrace, breeding material, research material, breeder's line, synthetic population, hybrid, founder stock/base population, inbred line (parent of hybrid cultivar), segregating population, mutant/genetic stock, market class and advanced/improved cultivar. Examples of cultivars include such cultivated varieties as Bintje, Russet Burbank, Eigenheimer, and Nicola.

As used herein, the terms "purebred", "pure inbred" or “inbred” are interchangeable and refer to a substantially homozygous plant or plant line obtained by repeated selfing and-or backcrossing.

As used herein, the term “molecular genetic marker” or short “marker” refers to an indicator that is used in methods for visualizing differences in characteristics of nucleic acid sequences. Examples of such indicators are restriction fragment length polymorphism (RFLP) markers, amplified fragment length polymorphism (AFLP) markers, single nucleotide polymorphisms (SNPs), insertion/deletion (INDEL) mutations, microsatellite markers (SSRs), sequence-characterized amplified regions (SCARs), cleaved amplified polymorphic sequence (CAPS) markers or isozyme markers or combinations of the markers described herein which defines a specific genetic and chromosomal location.

As used herein, the term "plant part" indicates a part of the potato plant, including organelles, single cells and cell tissues such as plant cells that are intact in plants, cell clumps and tissue cultures from which potato plants can be regenerated. Examples of plant parts include, but are not limited to, single cells and tissues from pollen, ovules, leaves, embryos, roots, root tips, tubers, anthers, flowers, fruits, stems shoots, and seeds; as well as pollen, ovules, leaves, embryos, roots, root tips, anthers, flowers, fruits, stems, shoots, scions, rootstocks, seeds, tubers, protoplasts, calli, and the like.

Unless expressly stated otherwise, the term "seed", as used throughout this specification, refers to the body from which a new plant develops (or kernel in some plants) containing the small embryonic plant enclosed in a seed coat covering, usually together with some stored food. This seed, referred to as botanical or "true" seed is the product of the ripened ovule of gymnosperm and angiosperm plants which occurs after fertilization and some growth within the mother plant.

As used herein the terms "vigour" and "vigorous" refer to the relative amount of above-ground or below-ground tissues of a plant, which relative amount may be more or less independent of one another.

The relative amount of above-ground tissues can be expressed as visual observations of the amount of foliage, in terms of plant height, number of stems, number of leaves assessed on ordinal scales, and/or in terms of fresh weight or dry matter. This trait has relevance during the first period of the plant growth cycle with an average duration of 100 days of cultivation (range 75 – 130 days) under normal growth conditions that prevail in North Western Europe during the season from May trough to August. Under these conditions, the plants of the present invention attain a weight of the foliage and shoots (above-ground plant) expressed in grams of fresh or dry weight which is at least 50%, more preferably at least 60%, 70%, 80%, 90% or more of the (essentially heterozygous) diploid breeding clone RH89-039-16, which – in turn - has 68% (range 44 – 86%) of the tuber yield of tetraploid potato varieties based on nine years of testing. Clone RH89-039-16 is available for reference purpose from Dr. Ir Ronald B.C. Hutten, Laboratory of Plant Breeding, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.

Alternatively, or in addition, the term vigour may be used in connection with a certain minimal tuber yield. The term "tuber yield", as used herein, will generally refer to a tuber yield expressed in grams of fresh weight which at harvest in plants according to the present invention is at least 30-50%, more preferably at least 60%, 70%, 80%, 90% or more of the (essentially heterozygous) diploid breeding clone RH89-039-16, which – in turn - has 68% (range 44 – 86%) of the tuber yield of tetraploid potato varieties based on nine years of field testing under conditions of a 100 days cultivation period with normal growth conditions that prevail in North Western Europe during the season from May trough to August. As indicated above, clone RH89-039-16 is available for reference purpose from Dr. Ir Ronald B.C. Hutten, Laboratory of Plant Breeding, Wageningen University.

Tuber yield, unless expressly stated otherwise, is based on the tubers formed from a seedling plant, in contrast to the tubers formed from a tuber-grown plant – which yield may be 50-200% more than the yield of a seedling.

Alternatively, or in addition, the term vigour may refer to a tuber yield expressed in grams of fresh or dry weight which at harvest is at least 30-50%, more preferably at least 60%, 70%, 80%, 90% or more of the diploid breeding clone RH89-039-16. This means that the tuber yield expressed in grams of fresh weight may (in plants of the present invention, reach levels of at least 200, more preferably at least 300, 400 or 500 grams per plant grown from seed in clay soil under normal North Western European summer conditions, and even 400, 500, 600, 700, 800 or more, such as 900 or 1000 grams of fresh tuber weight or more per plant when said plant is raised from tubers and grown in clay soil under normal North Western European summer conditions.

The term "a line comprising plants", wherein said plants are indicated as having a certain characteristic, such as tuber yield, should preferably be understood as referring to a line essentially consisting of plants having the said trait, while allowing for some biological variation.

The term "diploid" as used herein refers to a plant wherein each vegetative cell contains two sets of chromosomes (2x=2n, wherein n is the number of chromosomes). One set of chromosomes is donated from each parent.

The term "tetraploid" as used herein refers to a plant wherein each vegetative cell contains four sets of chromosomes (2x=4n).

The term "nematode resistance" as used herein refers to a plant in which a functional resistance gene has been introgressed that prevents the multiplication of at least one nematode population or isolate.

The term "disease resistance" as used herein refers to the ability to show more than 50 % reduction of the amount of diseased leaf surface or tuber volume, or the amount of multiplication of an insect or pathogenic microorganism including but not limited to *Streptomyces* spp, *Rhizoctonia*, Silver scurf and, *Phytophthora infestans*,

The term "herbicide tolerance" as used herein refers to a plant which shows less than 50% damage of the foliage than the usual damage upon the application of a specified dosage of herbicide.

The term "cold tolerance" as used herein refers to the average amount of leaf surface of a plant which shows frost damage, which damage in cold tolerant plants is less than the average damage observed at a temp of minus 3 °C for relatively cold sensitive reference varieties such as e.g. Caribe and/or Kennebec.

The term "drought tolerance" as used herein refers to a plant which shows less than average damage at limiting water conditions as compared to relatively drought sensitive reference varieties such as Caribe and/or Carlton.

The term "flooding tolerance" as used herein refers to a 50% lower proportion of affected tubers (anaerobic degradation) relative to a plant which shows average damage at flooding

The term "tolerance to wet rot" as used herein refers to a plant with the slightest level of resistance to *Erwinia* species (currently termed *Pectobacterium* spp.).

The term "tolerance to dry rot" as used herein refers to a plant with the slightest level of resistance to *Fusarium* species.

The term "salinity tolerance" as used herein refers to a plant which shows less damage than average at salinity conditions.

The term "growth rate" as used herein refers to the increase of plant biomass per unit of time.

The term "tuber development defects" (e.g. misshapen or damaged tubers)" as used herein refers to a plant which shows visual as well as internal malformations of tubers during plant development and/or tuber harvest.

The term "tuber yield" as used herein refers to the total weight of tubers of a plant or of a population of plants generally expressed in grams of fresh weight.

The term "tuber size" as used herein refers to the absolute width, height and length of a tuber. Beneficial tuber sizes include plant- average tuber sizes of about 80-160 grams per tuber. Mean tuber number for commercially valuable plants are about 8-12 tubers per plant.

The term "tuber skin colour" as used herein refers to the colour of the skin of a tuber after harvest as the result of anthocyanin accumulation in tuber skin tissues.

The term "eye depth" as used herein refers to the relative distance between the skin surface and the shoot primordium of a tuber.

The term "tuber shape" as used herein refers to the length / width ratio, to indicate the continuous variation from round, oval to long tuber shaps, as well as the height/width ratio, to indicate the continuous variation from cylindrical to the amount of flatness of a tuber.

The term "tuber flesh colour" as used herein refers to the colour of the interior of the tuber flesh after harvest as the result of the absence or presence of carotenoid compounds causing white or yellow flesh colour, respectively, as well as the absence of anthocyanins compounds causing red, blue, purple shades of flesh colour, irrespective of the presence of colour in patterns being partial or full.

The term "tuber taste" as used herein refers to the consumers appreciation by consuming a cooked potato tuber.

The term "tuber shelf life" and "tuber storage ability" as used herein are synonymous and refer to lack of changes in appearance of a tuber at storage.

The term "tuber dormancy period" as used herein refers to the time period between sowing and sprouting of a tuber, while sown at normal conditions.

The term "resistance to tuber dehydration" as used herein refers to a plant with shows less dehydration of the tuber than average at low humidity conditions (see shelf life).

The term "tuber starch content" as used herein refers to the starch weight over the total fresh weight of a tuber.

The term "tuber dry matter content" as used herein refers to the weight of the dry components of a potato divided over the total fresh weight.

The term "tuber cooking quality" as used herein refers to the continuous variation from firm to mealiness of a tuber after cooking.

The term "tuber frying quality" as used herein refers to the consumers appreciation of a tuber after frying.

The term "tuber chip making quality" as used herein refers to the consumers appreciation of a tuber after making chips.

The term "tuber uniformity" as used herein refers to the standard deviation of tuber traits, whereby a low standard variation refers to high uniformity and vice versa.

The term "cold sweetening resistance" as used herein refers to resistance of a tuber to produce glucose and/or fructose while stored at temperatures below 8 °C.

The term "tetraploid" as used herein refers to a plant wherein each vegetative cell contains four sets of chromosomes (4n). The terms "fertile" and "fertility" broadly refer to the capacity to reproduce, i.e. to conceive and to produce offspring, preferably fertile offspring. Thus, the term fertility refers to individuals that can be crossed to produce fertile offspring in that the flowers, when pollinated produce berries that contain seeds which when sown result in a plant that itself carries fertile flowers, etc. .

The term "fertile offspring" or "fertile seed" is defined herein as seed capable of growing into a flower-producing potato plant wherein flowers are male and female fertile. Thus, the term preferably refers to a plant or seed that when grown into a plant is capable of producing offspring as male and as female parent by virtue of the presence of fertile ovules and fertile pollen (i,e, both male and female flowers are fertile).

The term "fertile plant" is defined herein as a plant capable of producing fertile seed carrying berries. Preferably said berries each carry at least 5, more preferably at least 15-20, even more preferably at least 50 fertile seed, still more preferably between 50 and 500 seeds. Alternatively, a plant is said to be fertile when the number of seeds produced per berry is at least 10%, more preferably at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150% 200% or more of the number found in berries of the diploid breeding clone RH89-039-16. The term also, or alternatively, refers to plant lines having more than 25 %, preferably more than 90% fertile plants.

The term "self-compatible" refers to capacity to develop seeds in berries that are the result of self-pollination, self fertilization and producing fertile progeny.

The term "capable of being backcrossed/selfed/crossed and resulting in (self) fertile and self-compatible offspring" refers to the capability of producing one or more berries with seed as a result of backcrossing, selfing or crossing and wherein more than 60%, preferably more than 70%, 80%, 85%, 90% or more of said seed in said one or more berries is capable of developing into plants that are (self) fertile and self-compatible.

For other terms as used herein, reference is made to Allard, R. W. Principles of Plant Breeding, 2nd Edition, Wiley New York, 1999, and specifically to the Glossary therein.

Description of the preferred embodiments

The present inventors discovered that elite breeding lines for potato breeding can be successfully produced. Crossing of two elite breeding lines provides F1 hybrid seeds, such F1 hybrid seeds, when grown into plants, result in plants of superior agronomic performance. More importantly, all seeds are genetically essentially identical, meaning that, except for the occasional crossover or mutation event, all seeds are uniform.

The present inventors have discovered a method that allows the provision of elite potato breeding lines, which, when crossed mutually, provide uniform F1 hybrid potato seeds which, when grown into plants, result in plants having superior agronomic performance. The elite potato breeding lines may have complementary traits and, as a result of their homozygosity, the F1 plants grown from the F1 hybrid seeds are uniform, but when these F1 plants are subsequently selfed or cross-pollinated the resulting F2 plants will segregate for the various traits.

Thus, in contrast to the currently available true potato seeds (TPS) generated in the conventional ways of the prior art and harvested from berries in which every individual seed is genetically different from every other seed, the seeds obtained from crossing of the

potato lines and elite potato breeding lines in accordance with aspects of the present invention are genetically essentially identical.

The TPS varieties of the prior art are derived from crosses between selected but heterozygous parents and hence variability is expected in plants and tubers. Hybrid potato breeding as proposed herein differs from the conventional production of TPS in that the present TPS are the result of the crossing of two pure breeding lines, resulting in uniform cultivars, whereas TPS varieties of the prior art are the result of open pollination or crossing between two genetically non-homozygous and often tetraploid breeding lines resulting in non-uniform cultivars.

Not only are the presently provided plants novel in that they combine a number of properties, heretofore never attained together in a single potato line, they also allow for novel and very advantageous breeding methods. Hence, the plants of the invention are an intermediate in the production of novel potato cultivars, which cultivars can be produced by methods as disclosed herein. The advantageous breeding methods allow for the elimination of many of the drawbacks of conventional potato breeding as indicated in hereinabove including the elimination of the time-consuming selection of clones to arrive at a new potato variety and the possibility to provide uniform hybrid potato seed. In addition, the novel plants and methods also allow for the elimination of drawbacks related to clonal propagation and production, including the possibility to eliminate high transportation costs and the clonal persistence of pests and diseases.

Novel potato plants

Parental breeding lines

The present invention provides potato plants that are essentially homozygous, diploid, vigorous, fertile and self-compatible.

Preferably the plants of the invention have fixed within their genome nucleic acid sequences encoding for genes that confer to the plant an agronomically desirable trait. Such nucleic acid sequences may be introgressions or transgenes derived from potato germplasm with agronomically desirable traits, preferably from diploid germplasm.

- Essentially homozygous

In breeding, plants are propagated by sexual reproduction, usually by crossing of selected parents with desirable traits to produce improved progeny. Progeny inherit genes for both desirable and undesirable traits from both parents. Repeated selection of meritorious individuals from each generation that will serve as parents of the next will

conserve desired characteristics and eliminate undesirable ones. The ultimate parent in plant breeding is a purebred, a highly homozygous line that, when used in crossings with other purebreds results in offspring that is identical, both phenotypically and genotypically, within a single generation and between consecutive generations obtained from other crossings of the same parents. Purebreds, or pure inbreds, can be obtained by everal generations of selfing or inbreeding (crossing of close relatives, such as brother to sister plants or offspring to parent (backcrossing)) each successive generation showing an increased degrees of genetic uniformity, or homozygosity. The use of homozygous inbreds as breeding parents results in offspring that is identical, both phenotypically and genotypically, within a single generation and between consecutive generations obtained from other crossings of those parents. Hence, the pure inbreds can be used repeatedly to produce the same offspring plants.

In potato, selfing and inbreeding results in severe inbreeding depression, or the expression of deleterious recessive genes, which expression is revealed by loss of vigour and loss of fertility. Hence, it has hitherto been impossible to produce homozygous pure inbreds in potato since until the present invention, high levels of homozygosity in potato with sufficient vigour could not be attained. The present invention now, for the first time, provides plants having a level of homozygosity that is above 25%, and preferably above 75 % and that is combined with fertility, self compatibility and plant vigour.

- *Diploid*

The plants of the present invention are diploid plants, in contrast to the commercial potato varieties, which are tetraploid. It should be noted that in plants of the present invention higher ploidy levels are expressly envisaged, since the diploid stage is used primarily during the development of the breeding lines. Once the purebreds have been established, the lines may be made tetraploid. Hence, plants of the invention may also be tetraploid. Tetraploid plants can be produced from tetraploid cells or tissue cultures, which in turn may be produced by duplicating the genome of diploid cells or tissue cultures by methods known per se, for instance by using oryzalin as described by Barandalla *et al.* (Barandalla et al. Potato Research (2006) 49:143-154). Tetraploid plants may have the advantage that they provide higher yields.

- *Vigorous*

Although commercial tetraploid potato varieties are fertile and self-compatible and vigorous in that they grow well, do not produce deformed plants, or fruits, but manifest as healthy plants with a high tuber yields, their selfed progeny shows serious inbreeding

depression, that is, result in faint growing and low yielding plants with deformed tubers. Prior art diploid and essentially homozygous lines, such as those reported by Phumichai et al. as referred to herein, also grow only very poorly, and manifest as very weak and small plants, that have poor seed set. In fact, diploid potato plants resulting from inbreeding, and attaining homozygosity of at least 25 % or 50% on the alleles are invariably weak growers.

The plants of the present invention are not weak and frail, and although they need not necessarily be as vigorous as present day commercial tetraploid varieties, they do not show inbreeding depression. Hence, within the context of the present invention, they are vigorous. Tuber formation exemplifies the vigour of the plants of the present invention. Although all commercial (tetraploid and seed-tuber derived) potato varieties produce large tubers, or at least tubers of sufficient size, prior art diploid lines and essentially homozygous plants produce only small tubers, which are, for instance, of no use in the production of potato chips or French fries. Prior art diploid lines and essentially homozygous plants produce also very few tubers such that the yield of tubers per plant is at least ten times lower than that of heterozygous diploids plants and commercial tetraploid potato varieties. In contrast, the plants of the present invention exhibit beneficial tuber formation characteristics, including tubers of beneficial size profile, and a tuber yield that may be at least 50% compared to the diploid heterozygous plants used as donors in the examples as described herein (D1, D2, and/or D3).

As was found herein, the tuber yield of the diploid F1's was on average 443 grams per plant, with values as high as 600 and 634 grams.

Backcrossing with the F1: "IVP08-06" yielded on average 155 and 209 grams, while backcrossing with the F1: "IVP08-07" yielded on average 209 and 259 grams.

The "pseudo F2's" yielded on average 172 and 332 grams per plant, and occasionally produced higher than the diploid F1's.

The F2's yielded on average 69 and 252 grams per plant. Several plants overlap with the yield values of the diploid F1s.

- *fertile*

The plants of the present invention – although essentially homozygous – are also fertile. That means that they can be used in breeding programs because their offspring will also be fertile.

- self compatible

The plants of the present invention are self compatible. This feature is introduced in order to allow the selfing of plants and the production of homozygous lines.

The preferred basis of the self compatibility is derived from the dominant S locus inhibitor gene (*Sli*) (Hosaka and Hanneman, 1998, Euphytica, 99: 191–197).

The essentially homozygous potato plants of the present invention have as an advantage that they can be crossed with other essentially homozygous potato plants whereby the hybrid offspring of said cross is essentially genetically uniform and will not segregate.

The present invention, in a preferred embodiment, provides essentially homozygous, diploid, vigorous, fertile, and self-compatible potato plants having agronomically desirable traits.

The novel potato plants of the invention are capable of:

- being backcrossed to an essentially homozygous recurrent parent and resulting in (self) fertile and self-compatible offspring;
- being selfed (self-fertilization of 1 offspring individual) and resulting in (self) fertile and self-compatible offspring;
- being mutually crossed (fertilization between 2 offspring individuals) and resulting in (self) fertile and self-compatible offspring.

Plants according to the present invention include the line with breeders reference AGVD1, representative seeds of said line having been deposited on 23 October 2009 with the NCIMB, Aberdeen, Scotland under accession number NCIMB 41663 and breeders reference AGVD1, which plant represents an F2 obtained by crossing a plant of parent IVP07-1001/4 (see Example 1), which is an essentially homozygous, diploid, fertile and self-compatible but non-vigorous potato line with a plant of parent D1, which is an elite diploid breeding clone that is essentially non-homozygous, and is not self-compatible, but which has the desirable agronomic trait of yellow flesh color and exhibits good cooking quality in that the flesh is not greying after cooking, and selfing the resulting F1 offspring plants. It is estimated that AGVD1 is about 50% homozygous, meaning that about 50% of the loci for which the parents differ are homozygous.

Plants according to the present invention also include the line with breeders reference AGVD2, representative seeds of said line having been deposited on 23 October 2009 with the NCIMB, Aberdeen, Scotland under accession number NCIMB 41664 and under breeders reference AGVD2, which line represents an (F1 x F1) line (or pseudo F2) obtained by crossing a plant of parent IVP07-1001/4 as indicated above with a plant of parent D1 as indicated above, and mutually crossing individual F1 offspring plants, that share the IVP07-1001/4 parent but have different individuals of the D1 parent. It is

estimated that AGVD2 is about 25% homozygous for the loci for which the parents differ.

Plants according to the present invention also include the line with breeders reference AGVD3, representative seeds of said line having been deposited on 23 October 2009 with the NCIMB, Aberdeen, Scotland under accession number NCIMB 41665 and under breeders reference AGVD3, which line represents a BC1 population obtained by crossing a plant of parent IVP07-1001/4 as indicated above with a plant of parent D1 as indicated above, and backcrossing the resulting F1 offspring plants with the IVP07-1001/4 parent. It is estimated that AGVD3 is about 75% homozygous for the loci for which the parents differ.

Plants according to the present invention also include the line with breeders reference AGVD17, representative seeds of said line having been deposited on 5 October 2010 with the NCIMB, Aberdeen, Scotland under accession number NCIMB 41765 and under breeders reference AGVD17, which line represents an F3 population obtained by crossing a plant of parent IVP07-1001/4 as indicated above with a plant of parent D2 as indicated above, and backcrossing the resulting F1 offspring plants with the IVP07-1001/4 parent, followed by two steps of selfing. It is estimated that AGVD17 is > 80% homozygous for the loci for which the parents differ.

A plant of the present invention can very suitably be used as a recurrent parent wherein additional agronomically desirable traits are introgressed by crossing, or introduced by other means such as by transgenic means. Once a number of agronomically desirable traits have been stacked into an essentially homozygous, vigorous, diploid, fertile and self-compatible plant according to the invention, that plant can be used as an elite breeding line for the production of commercial F_1 hybrid potato seed. Likewise, a second elite breeding line can be provided that can serve as the parent of opposite sex so that both plants can be crossed to produce the commercial F_1 seed of a novel potato variety.

The breeding of uniform hybrid seed depends on a number of technical conditions. Prerequisites to achieve uniform hybrid seed are that i) an initial introgression crossing can be made between an essentially homozygous, diploid, fertile and self-compatible plant and a plant that acts as donor for an agronomically desirable trait, that ii) a backcross can be produced between the offspring of that initial crossing and the essentially homozygous, diploid, fertile and self-compatible plant, and that iii) the offspring thereof can be selfed. Potato lines AGVD1, AGVD2 and AGVD3 exhibit these characteristics and individual plants of these lines can be used in additional introgression crossings, backcrossings and selfings to arrive at the elite breeding lines suitable for use as

parents for commercial F1 hybrid seed that can be grown into hybrid potato plant of a new potato variety.

A highly preferred plant according to the present invention is a plant of line AGVD2 or an offspring plant thereof having all of the physiological and morphological characteristics of the plant of line AGVD2.

Another line deposited as example of embodiments of the present invention is AGVD17 (NCIMB 41765, deposited 05 October 2010). The advantage of AGVD17 is that it is a highly homozygous line (> 80% homozygous) combining vigour and fertility with a tuber yield of on average 300 grams (fresh weight) per plant. Individual plants of related populations even showed tuber yields of 500 grams per plant. These data were generated from field grown plants that were raised from seedlings. Potato plants raised from clones generally yield tuber weights that are 50 to 100% higher than plants raised from seedlings. Hence, the tuber yield expressed in grams of fresh weight from plants as claimed herein – when clonally propagated and raised from tubers and grown in clay soil under normal North Western European summer conditions – is expected to reach levels of at least about 1000 grams fresh weight per plant.

Thus, line AGVD17 demonstrates in particular the possibility to intercross individual elite lines of the present invention (i.e. that are self-compatible, fertile, and essentially homozygous) to arrive at the hybrid seed that is the aim of the present invention. Hence, another highly preferred plant according to the present invention is a plant of line AGVD17 or an offspring plant thereof having all of the physiological and morphological characteristics of the plant of line AGVD17.

Hybrids

The hybrid plants of the invention are produced by a method of the invention as exemplified in Figure 1. The hybrid plants of the invention are produced by crossing two essentially homozygous elite potato parental breeding lines comprising among them a number of agronomically desirable traits selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, cold sweetening resistance and resistance to tuber dehydration. In addition to these agronomically desirable traits, the parental breeding lines may exhibit such characteristics as desired in specific use markets such as desirable tuber skin color, desirable tuber shape, desirable tuber flesh color, desirable tuber taste, desirable tuber starch content, desirable tuber dry matter content, desirable tuber cooking quality, or

desirable tuber frying quality. In addition to such market-specific characteristics, the parental breeding lines, when crossed, preferably result in hybrids that exhibit such beneficial traits as beneficial growth rate, beneficial tuber yield, beneficial tuber size, beneficial tuber storage ability, beneficial tuber dormancy period, beneficial tuber shelf life, beneficial tuber chip making quality, and beneficial tuber uniformity.

The present invention now provides a set of potato plants or a set of potato plant lines, which plants or plant lines are: diploid (2n); essentially homozygous (preferably more than 60% on all alleles); self compatible; fertile; and vigorous. Hitherto it has proven impossible to provide the combination of all these features in a single potato plant (line). The set of potato plant of the invention can be used to produce novel varieties or cultivars.

The invention particularly relates to a set of at least 2 diploid (2n), essentially homozygous (preferably more than 60% on all alleles), self compatible, fertile, and vigorous plants that are crossable to produce hybrid varieties according to the invention.

Novel method of potato seed breeding

The provision of the novel potato plants now provides for the first time the attainment of the method of the invention.

The present inventors have generated potato plants that are essentially homozygous, diploid, vigorous, fertile and self-compatible and having fixed within their genome nucleic acid sequences encoding genes that confer to the plant an agronomically desirable trait derived from suitable diploid donor lines. The said nucleic acid sequences are the result of introgressions from potato germplasm having agronomically desirable traits. Conventionally, at least in most species other than potato, homozygous plants can in principle be obtained in as little as two generations by doubling of monohaploids generated from unfertilised generative cells such as egg or pollen cells by methods known as doubled haploid (DH) technology. This is not possible in potato, as the resulting plants have hitherto shown to be infertile, due to - what is considered – expression of recessive deleterious genes. The DH method was extensively tested by the present inventors, but was found unsuccessful.

The present inventors used the strategy of repeated selfing, backcrossing and crossing of potato germplasm of a single self-compatible potato line and one or more self-incompatible potato lines harbouring agronomic traits. The homozygous plants of the present invention were obtained by continuous selfing of diploid potato plants and selection for fertile offspring (besides the inherent selection for self-compatibility). This is an entirely new approach, as in the prior art methods, plants are obtained by selection for

agronomical traits, such as tuber formation or general growth characteristics of the offspring plants, not by selection for fertile offspring from selfings. Moreover, and unlike the prior art methods the procedures are performed using diploid plant lines, instead to tetraploid plants. Unexpectedly, this radically new approach proved successful for obtaining increasingly homozygous, diploid offspring plants, that were self-compatibel and fertile. In addition, when (out)crossed with plants of diploid lines harbouring beneficial traits, such as resistance or white tuber color, these traits could be introduced into the essentially homozygous germplasm without loss of self-compatibility and fertility.

In essence the method for obtaining the plants is as follows:

Starting point is a diploid self-compatible potato line (examples of suitable lines have been deposited under depositors reference AGVD 1, AGDV 2, AGDV 3 and AGVD17). In general a line being self-compatible due to the presence of the *Sli* gene may be used as starting point. Plants of this diploid self-compatible line are selfed and seeds obtained from seed-carrying berries are sown and grown into offspring plants. Offspring plants carrying flowers are again selfed and seeds obtained from seed-carrying berries are again sown and grown into offspring plants. No, or at least little, attention is paid to the agronomical appearance of the plants at this stage. They may look withered and frail, with only a few flowers. Nonetheless, the selfing is repeated at least three times, but preferably 4 or 5 or even 6-8 times. In this manner a line of essentially homozygous, fertile, self-compatible, and diploid potato plants is obtained, essentially by selfing and selecting for plants that produce fertile offspring.

Next, an agronomically desirable trait is added to the essentially homozygous, fertile, self-compatible, diploid potato genotype by crossing a plant of that line with a plant of an unrelated essentially heterozygous diploid potato line, preferably a line containing an agronomically desirable trait. This essentially heterozygous line (which term merely reflects that the line is not an essentially homozygous line as used herein) functions as a donor for an agronomically desirable trait, in this case vigour.

The resulting hybrid is selfed and/or backcrossed to the self-compatible line while selecting for higher vigour. In this way, the vigour of the essentially homozygous, fertile, self-compatible, diploid potato plant line is enhanced and at the same time the line is agronomically improved. These steps will thus result in a vigorous fertile homozygous self-compatible diploid potato: The vigour may further be enhanced by crossing with other essentially heterozygous diploid potato lines, which additional lines serve as donors for additional agronomically desirable traits, followed by selfing and/or backcrossing to to the self-compatible line while selecting for these additional traits, which additional steps will result in the provision of diploid potato breeding lines.

In order to produce elite diploid potato breeding lines, the breeding value of the breeding lines is further enhanced by intercrossing, selfing and backcrossing while continuous selecting for the agronomically desirable traits provided to the breeding line, and by establishing lines that exhibit good combining abilities with other lines. Such combining abilities are evaluated by the use of experimental crosses.

Finally, hybrid potato cultivars can be produced by crossing individual elite diploid potato breeding lines that exhibit good combining abilities. The seeds resulting from this cross of homozygous parents are hybrid true potato seeds that are uniform.

Using the methods of the present invention, generally, around 7 to 8 selfing cycles will result in essentially homozygous plants, but the skilled artisan will understand that more generations of selfing may also be used. Also fewer generations, such as six generations may be used to provide essentially homozygous plants of the present invention. The number of backcross generation can even be reduced to three to reach more than 95% homozygosity when applying molecular genetic markers.

Essentially, the novel method of producing hybrid potato cultivars provides for the possibility to produce a new potato variety within about 5 years. A detailed process for the production of hybrid potatoes starting from AGVD17 (F3 line) is provided in Example 5. This line is >80% homozygous such that very few selfings are needed to obtain a plant that is about 95%-100% homozygous. In addition AGVD17 is already vigorous as defined herein.

The present invention now provides a method for producing a genetically homogenous or uniform true potato seed (TPS). This method is *inter alia* useful in a method of preventing transmittal of virus infections between potato generations, but also for overcoming many of the other problems associated with classical potato breeding, "seed" potato production and transport and cultivation. Moreover, the present parental material for production of hybrid seed can be maintained in the form of seed, and not in the form of vegetative material as is required by traditional TPS production.

The present invention provides in one aspect a method for producing a hybrid potato cultivar comprising the use of the deposited plant material provided herein as a starting material.

Alternatively, other essentially homozygous, diploid, fertile, vigorous and self-compatible plant lines different from the deposits referred to herein may be developed and the production of a hybrid potato cultivar can commence from that early stage level by using the teaching provided herein since the present inventors have demonstrated that this

route, although laborious, is successful. Such an alternative breeding material is also vigorous, essentially homozygous, diploid, fertile and self-compatible.

As a first step, such an early stage development may comprise the provision of a plant of a first cultivated potato species (*Solanum tuberosum*) or a related crossable species (generally a tuber bearing *Solanum* species).

Next, said plant is developed into a line of self-compatible diploid potato plants, preferably a *Solanum tuberosum*. Diploid potato is usually self incompatible. Self-compatibility may suitably be introduced from related species (see for instance Hermsen, J.G.Th. 1978, Euphytica 27, 1-11).

The resulting line of self-compatible diploid potato plants is then developed into a homozygous fertile self-compatible diploid potato line. Homozygous diploids can be obtained by doubling haploids (Uijtewaal et al., 1987, Theor. Appl. Genet. 73, 751-78) by anther culture (Jacobsen and Sopory, 1977, Theor. Appl. Genet. 52, 119-123), by ovule culture or by repeated selfings (Phumichai & Hosaka, 2006, Euphytica 149, 251-258). The latter method was used for producing the homozygous fertile self-compatible diploid potato line that formed the basis of the vigorous plants as described herein.

Next, the homozygous fertile self-compatible diploid potato line is developed into a vigorous fertile homozygous self-compatible diploid potato line. The vigour may be enhanced by crossing with other diploid potato plants, selfing and backcrossing while selecting for higher vigour. It must be noted that high populations are needed and that even for some unrelated essentially heterozygous diploid potato lines that are used as donor of the agronomically desirable trait, very few self compatible plants may be found in a population of 1000 plants. Generally, such attempts are terminated, and only when about 10-50% of the offspring plants is self-compatible (such as in the case of D2 in Example 2 below) the donor line exhibits such good general combining ability that a successful introduction of the agronomical trait into the self-compatible lineage is foreseen.

Next, the vigorous, fertile, homozygous, self-compatible, diploid potato line is developed into a diploid potato breeding line. Most preferably, at least two separate vigorous fertile homozygous self-compatible diploid potato lines are produced and at least two distinct diploid potato breeding lines are developed. Such breeding lines comprise a high number of agronomically desirable traits. Agronomically desirable traits are introduced by crossing with other diploid potato plants, selfing and backcrossing while selecting for these traits. This breeding program may comprise testing for disease, such as whether the shoots or tubers are free of disease, or whether they are resistant for disease.

Next, the diploid potato breeding lines are developed into elite diploid potato breeding lines. This is accomplished by enhancing the breeding value by intercrossing,

selfing and backcrossing while continuously selecting for agronomically desirable traits. At this stage experimental crosses are performed to evaluate general combining abilities. The diploid elite lines can now also be developed into tetraploid (4x) elite lines by methods known per sé, such as by chemical treatment with e.g. colchicine, or by "spontaneous" doubling of the genome using tissue culture.

Finally, potato hybrid cultivars are produced from these elite diploid or tetreploid potato breeding lines by crossing of good combining breeding lines. The seeds obtained are hybrid true potato seeds that are uniform.

The method of the invention for producing a potato hybrid cultivar comprises the steps of producing a diploid potato breeding line. Said method comprises the steps of:

(a) providing a first potato plant, which first potato plant is a plant of a diploid, self-compatible and essentially homozygous potato line, preferably a plant of a vigorous fertile homozygous self-compatible diploid potato line, more preferably a diploid potato breeding line;

(b) providing a second potato plant of a potato line that may have any level of ploidy, preferably diploid, and/or any level of homozygosity wherein said second potato plant serves as a donor for an agronomically desirable trait which trait is to be introgressed into said essentially homozygous line of said first potato plant or at least in an offspring plant resulting from a crossing between said first and second plant, wherein said trait is selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, rooting ability, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, growth rate, tuber development defects (e.g. misshapen or damaged tubers), tuber yield, tuber size, tuber skin color, eye depth, tuber shape, tuber flesh color, tuber taste, tuber storage ability, tuber dormancy period, tuber shelf life, resistance to tuber dehydration, tuber starch content, tuber dry matter content, tuber cooking quality, tuber frying quality, tuber chip making quality, tuber uniformity and cold sweetening resistance.

Following the provision of these two plants, the method then comprises the step of:

(c) cross-pollinating said first and second potato plant such that one of said parent plants produces seed-carrying fruits (berries) and collecting said seeds from said fruits to provide a hybrid offspring seed.

Seeds are then grown to plants and the plants that comprise the agronomically desirable trait from the donor plant are selected, either directly or following additional

selfing and backcrossing steps, as is well known in the art of plant breeding. In these methods markers may be used to assist in the breeding process.

The hybrid offspring seed is preferably grown into a plant that is subsequently backcrossed a plant to the recurrent parent line

The following steps are not essential to either of the above methods. However, these additional steps may either provide for the production of consumption tubers by growing the seeds of the invention and/or may provide for the production of new homozygous, self-compatible, diploid elite breeding lines that can serve as parents for the inclusion of yet further traits into an offspring generation. The plants of the invention are fertile and vigorous. These additional benefits are attained by the following further steps of:

(d) growing said offspring seed into a hybrid offspring potato plant, and

(e1) harvesting tubers from said hybrid offspring potato plant to provide tubers carrying said trait, or

(e2) selfing said hybrid offspring potato plant or backcrossing said hybrid offspring potato plant to a potato plant of said line of said first potato plant to provide further offspring plants carrying said trait and wherein said of offspring plants are increasingly homozygous following each backcrossing or selfing step.

Preferably said second potato plant used in the method described above is a hybrid offspring potato plant as defined in step (d) above and obtained by the method as described above. More preferably, said second potato plant used in the method described above is a hybrid offspring potato plant as defined in step (e2) above and obtained by the method as described above.

Preferably, said step (e2) comprises from 2 to 7 backcrosses using a plant of line AGVD1, AGVD2, AGVD3 or AGVD17 as the recurrent parent and testing offspring plants for the presence of the trait (e.g. in the form of the homozygous or heterozygous presence of alleles responsible for the trait), followed by one or two (or more) selfings, to provide an essentially homozygous plant that is genetically equal to a plant of population AGVD1, AGVD2, AGVD3 or AGVD17, but in which alleles responsible for the trait are heterozygously, preferably homozygously present. This plant has at least one additional allele not available in population AGVD1, AGVD2, AGVD3 or AGVD17.

The above method now provides a breeding process for the production of homozygous breeding lines or elite breeding lines based on the background of population AGVD1, AGVD2, AGVD3 or AGVD17, to which background successively additional alleles are added, thereby providing elite breeding lines wherein desirable traits are stacked.

The method of the present invention now for the first time provides for a method for preventing transmittal of virus infections between potato generations while at the same time also solving many of the other problems of the prior art related to breeding of new potato varieties and commercial production of potatoes for consumption. This has become possible through the provision of true potato seed, i.e. botanical seed, which seed can be grown to a potato plant that produces tubers for consumption, and which seeds are from a cross in which at least one of the parents is a plant of a diploid, self-compatible and essentially homozygous potato line, preferably said seeds are from a cross wherein both of the parents are plants from a diploid, self-compatible and essentially homozygous breeding line.

An important advantage of this new method is that, in this way, the transmittal of infection with a virus that is not spread through the gametes and seeds can effectively be prevented.

The present inventors now provide a method for further breeding for homozygous lines that are easy to handle, and which exhibit improved fertility and plant vigour when compared to prior art inbreds, while maintaining the self-compatibility and high level of homozygosity. It will be appreciated that – just as in tomato - a 100% homozygosity is not always needed to provide for an effective breeding program. This is not a problem as long the self-compatibility is maintained.

The genetic transfer of agronomically desirable or beneficial traits into a homozygous potato line has now for the first time been successfully shown.

Based on the means and methods provided herein, the skilled person will be able to successfully produce homozygous potato breeding lines based on his own germplasm. In addition, the skilled person will appreciate that means and methods are provided by which he is able to, for instance, stack additional traits into the presently provided self-compatible and essentially homozygous lines in order to establish a population of lines that are enriched for agronomically desirable traits and that can serve as germplasm for commercial breeding in their own right.

EXAMPLES

Example 1. General description of the breeding procedure and pedigrees.

Many attempts were made to generate homozygous, fertile, self compatible diploid genotypes of potato without success. These attempts included the formation of doubled haploids, and the subsequent screening for fertile flowers. Several methods were employed like anther culture to regenerate haploid and eventually double haploid homozygous plants, repeated selfings to gradually increase the level of homozygosity in existing diploid germplasm and prickle pollination by using S. phureja as pollen donor to induce embryo formation from unfertilized egg cells. Especially the latter method was done at a large scale, by analyzing more than two million offspring but without the desired success. None of these tests resulted in vigourous self compatible plants.

An essentially homozygous potato line was obtained by multiple generations of selfing of plants of a line derived from public germplasm which plants have the self-compatibility controlling *Sli* gene (Phumichai et al. Euphytica (2006) 148: 227–234). These plants, designated "H" or IVP07-1001/4 herein, grew poorly, had very few flowers and each berry contained only few seeds. Moreover, the plants were very small (max 60 cm). The plants produced almost no tubers and those that formed were very small (average tuber weight about 5 grams/tuber). In all, the plants were of no value to commercial potato breeding, as they exhibited a plant weight and tuber yield that was less than 20% of that of diploid breeding clone RH89-039-16. The plants merely contained homozygous alleles on many of the loci in their genome, were self-compatible and diploid.

In the summer of 2008 plants of this IVP07-1001/4 line were crossed with different diploid donor lines (D1, D2 and D3, See for complete pedigree figure 2). D1 is early, has long sized oval tubers, light yellow flesh, and good cooking fries making quality and good cooking quality and was used as male parent (Table 6). The resulting progeny is referred to as F1(D1) in this example. Other F1 offspring plants were obtained in the similar way but using donor D2 as a female parent. D2 is an elite diploid breeding clone that is essentially non-homozygous, and is not self-compatible, but which has the desirable agronomic trait of early cooking, oval tubers, yellow flesh and good cooking chips making quality). This resulted in lines designated as F1(D2).

The above F1's were (D1) was selfed to produce an F2(D1). This line was deposited under breeders reference AGVD1. It is estimated that AGVD1 is about 50% homozygous, meaning that about 50% of the loci for which the parents differ are homozygous.

F1(D1) was crossed with F1(D2) to produce a pseudo F2. This line (F2(D1/2)) was deposited under breeders reference AGVD2. It is estimated that AGVD2 contains about 25% homozygous alleles originating from IVP07-1001/4.

The F1's were(D1) was also backcrossed with IVP07-1001/4 to produce a BC1's line. This line (BC1(D1) and BC1(D2)) was deposited under breeders reference AGVD3. It is estimated that AGVD3 is about 75% homozygous for the loci for which the parents differ.

Other F1 offspring plants were obtained in a similar way by using donor D3 as female parent. D3 is a diploid breeding clone with yellow flesh and with the resistance genes R3, H1, Gpa2, RXadg. This resulted in a line designated F1(D3).

Similarly as described above, F1(D3) was selfed to generate an F2(D3) and backcrossed with the Sli gene donor to generate BC1(D3) offspring.

F1(D1) was crossed with F1(D2) to produce a pseudo F2. This line (F2(D1/2)) was deposited under breeders reference AGVD2. It is estimated that AGVD2 is about 25% homozygous for the loci for which the parents differ.

F1(D1) was also backcrossed with IVP07-1001/4 to produce a BC1 line. This line (BC1(D1)) was deposited under breeders reference AGVD3. It is estimated that AGVD3 is about 75% homozygous for the loci for which the parents differ.

The BC1's were selfed in a winter nursery resulting in BC1S1 populations. Next summer (2010) the F2's, the pseudo F2's and the BC1S1's were sown in seed beds in a nursery and transplanted in small peat pots. In June 2010 the seedlings were transplanted to the field at a clay and at a sandy soil in The Netherlands. In total more than 15000 plants were grown from seedlings and selected for self compatibility and for good tuber quality and yield. Most F2 and BC1S1 populations showed a very low frequency of self-compatibility. Rare plants with reasonable good berry- and seed set were selected for progeny testing without further agronomic evaluations. Only two F2 populations, originating from the D2 x H cross, showed about 25% self compatibility. For each of the most self-compatible populations maximally fifteen plants with the best tuber quality and quantity were selected for progeny testing.

Plants of the pseudo F2's showed much more vigour than the other populations, probably as these plants do not harbour any locus with homozygous alleles of the donor parents. One of these populations showed more than 50% self-compatibility. Similarly as described above, also maximally fifteen plants of the most self-compatible populations were selected for progeny testing.

In addition, crosses were made with self-compatible plants from the field as pollen donor on two to twelve plants of the donors D1-D19 (Example 3). After evaluation

of the tuber quality and quantity only crosses with selected plants or their full sibs, in case of lack of sufficient numbers of crosses, were selected for progeny testing.

In this way, in each generation, plants are selected for self-compatibility and good agronomic performance. Seeds of the selfed berries of these selected plants are collected for further testing in order to generate homozygous lines. In addition, crosses of the selected plants with other diploid donors or other breeding lines are made to enhance the genetic variation. These F1 seeds are heterozygous and several rounds of inbreeding are needed to generate homozygous offspring with the desired traits.

In this way two generations of selections, selfings and crosses can be done per year. This process is even further enhanced by exploiting marker aided breeding by using diagnostic markers for specific traits and random markers to select for the recurrent parent genome. When elite parents have been obtained, derivatives of these lines can be made by two to three generations of backcrossing and two generations of selfing.

Example 2 Breeding self compatible diploid potato.

1. Introduction

A common characteristic of diploid potato is self-incompatibility (SI); upon self-fertilisation no vital progeny is generated. The S-locus inhibitor gene (*Sli*) originating from *Solanum chacoense* has been described (*Euphytica* 99,191-197, 1998) to inhibit this self-incompatibility and rendering diploid potato self-compatible (SC). Upon repeated selfings, close to homozygous self-compatible diploid potato clones have been generated (Genome 48, 977-984, 2005). The agricultural quality of these clones was very poor indeed reflecting a poor germination, plant growth, tuber quality and tuber yield.

2. Experiments

2.1 Materials

In order to develop self-compatible potato clones with much higher level of agricultural traits we crossed the *Sli* gene donor with various potato clones, designated: IVP97-079-9, IVPAA-096-18 and SH83-92-488. These clones are available at Dr. R.C.B. Hutten, Laboratory of Plant Breeding, Wageningen University, The Netherlands. As used herein, these clones are designated as clones D1, D2 and D3 resp. The progenies (F1) were selfed and backcrossed to the *Sli* gene donor generating F2 and BC1 populations, respectively. The BC1 was selfed to generate BC1S1 populations. All these crosses and

self fertilisations were performed without any other selection than self compatibility of the plants.

2.2 Evaluation of progenies in a wintertrial

In a pilot experiment several random BC1 and F2 progenies were grown in a heated greenhouse with 16 hours artificial light in while outside temperatures were below zero for almost three months. We evaluated the self-compatible % as the number of plants with berries after hand pollination and the yield as the weight of tubers per plant (Table 2).

Results

The *Sli* donor is self-compatible (SC) and most of the F1 and BC1 plants were also self-compatible, while a minority of the F2 plants was self-compatible. The *Sli* donor showed an extremely low tuber yield, while the BC1 and one F2 was about 50% of the F1 tuber yield. The tuber yield of some BC1 plants was close to that of the F1 plants and some (unselected) F2 plants were better than the weakest F1 plants.

Table 2. Some characteristics of winter grown plants of a diploid potato breeding program to introduce the *Sli* gene.

	# plants	compatibility	yield (gram per SC plant)	
		frequency (%)	average	variation
Sli donor	2	100	22*	0 - 43
F1 (HxD3)	9	89	443**	258 - 634
BC1 (Hx(HxD1)	49	81	206**	0 - 524
BC1 (Hx(HxD2)	64	72	237**	28 - 543
F2 selfed (HxD1)	23	22	252**	131 - 376
F2 selfed (HxD2)	20	25	69**	0 - 201

* from tuber

** from seedling

2.3 Evaluations of progenies in the field

In the summer of 2010 progeny plants of F2 and BC1S1 populations were tested in the field to select self-compatible progeny plants with good agricultural performance (Table 3).

Table 3. Breeding scheme to introduce self-compatibility (SC) into diploid potato. In bold the most promising materials are indicated.

P	F1 (HxD)	F2: selfed (HxD)				BC1S1: selfed (Hx(HxD))			
parents	plants (DxH)	Populations tested	plants in the field	self compatible plants	plants selected*	populations tested	plants in the field	self compatible plants	plants selected
	#	#	#	#	#	#	#	#	#
H = *Sli* donor									
D1	33	15	2460	± 50	7	38	2521	< 10	1
D2	45	29	***5598***	***> 500***	***52***	24	1380	< 10	1
D3	8	8	1602	± 10	7				

* sufficient seedset

Results

We tested 52 F2 populations comprising 30 to 840 plants and 62 BC1S1 populations comprising 1 to 390 plants, in total 9660 F2 plants and 3901 BC1S1 plants resp. Most of the populations showed a weak plant growth and a low frequency of self-compatibility. Only two F2 populations derived from the D2 parent showed about 25% self compatible plants. The best plants of these populations were selected on the basis of tuber yield and tuber quality.

3. Conclusion

Only two out of the 52 F2 populations and none of the BC1S1 populations showed a reasonable level of self-compatibility. However, unexpectedly, the desired plants could be obtained, starting from material that would otherwise have been neglected by breeders. The main differences of the plants of the invention and those of the prior art are given in Table 4 below.

Table 4. Characteristics of the parental potato lines of the present invention proposed for the production of hybrid (true) potato seed.

	Vigour	**Ploidy**	**Tuber yield**	**Fertility**	**Self-compatibility.**
Sli-gene donor (Phumichai et al. Euphytica (2006) 148: 227–234).	low	2n	low	low/ intermediate	high
Diploid trait Donor (e.g. D1)	intermediate	2n	intermediate	intermediate	low
Commercial variety	high	4n	high	high	low
Lines of present invention	Intermediate /high	2n	Intermediate /high	low/ intermediate	high

Example 3 Development of a molecular marker platform

Introduction

Numerous marker systems are available to support genetic research. For the present invention a molecular marker platform in potato was developed and used to evaluate the level of homozygosity versus heterozygosity in diploid potato.

Selection of most informative sequences for SNP assays

In order to analyse SNPs in potato, the Illumina standard GoldenGate protocol (Shen et al. 2005. Mutation Research 573:70–82) has been used previously (Anithakumari et al. 2010. Mol. Breeding, 26:65-75). This platform comprises 384 SNPs and was later expanded with another 768 SNPs. These 384 and 768 SNPs have been developed using EST sequences, which represent the mRNA without intron sequences. Since the present inventors have access to the first draft sequence of the potato genome (www.potatogenome.net) it was possible to compare the mRNA with the genomic DNA sequence including the intervening sequences. This comparison with the potato genome sequence was performed with the Basic Local Alignment Search Tool (BLAST; Altschul et al. 1990. J. Mol. Biol. 215: 403-410). This resulted in 538 and 1681 hits, respectively. The occurrence of multiple BLAST hits was considered to reflect paralogs or intron spanning sequences. These were removed from the initial collection of SNPs that were first developed for GoldenGate analysis (Anithakumari et al. 2010). This resulted in 279 and 453 unique BLAST hits representing the occurrence of only one copy in the potato genome sequence of *Solanum tuberosum* Group Phureja DM1-3 516R44 (CIP801092) as known hitherto (Potato Genome Sequencing Consortium, www.potatogemome.net).

From the total of 732 SNPs with unique BLAST hits only a few (31 cases, equally distributed over the 384 and 768 set) were omitted due to high level of sequence mismatches (3-6 SNPs within 101 bp). One mismatch should be expected and a second SNP was tolerated to be included in the SNP-spanning sequences selected.

For the present SNP design the minimal sequence length was set at 101 bp. Therefore 81 sequences were removed as they were shorter. The resulting 553 sequences have a length of at least 101 bp. A total of 100 sequences were then removed for having incomplete matches of only 35 up to 94 nucleotides within a scaffold of a doubled monoploid (DM-3 516R44; www.potatogenome.net). Also removed were sequences with introduced alignment gaps and sequences having other potential mismatch problems.

Though twenty-five sequences did not meet the criteria described above, they were kept as these were were known to be highly informative in the GoldenGate SNP assays. These SNPs even allowed to discriminate between the five genotype classes that occur in tetraploids (Voorrips et al, EAPR-EUCARPIA congress "Potato Breeding after completion of the DNA Sequence of the Potato Genome", 27-30 June 2010, Wageningen, the Netherlands, e-publication at http://edepot.wur.nl/143559, pg 42).

Finally, 515 sequences were selected. The final selection of 100 sequences was based on the known map position of 237 markers (out of the 515) on the potato genetic map (Anithakumari et al, 2010 and Van Os et al. Genetics 173: 1075-1087, 2006). The selected markers were evenly distributed over the 12 potato chromosomes including the most distal telomeric as well as centromeric markers and with a preference for SNPs that were heterozygous in clone RH (RH89-039-16; www.potatogenome.net), because clone RH is a progenitor of the material used in the present research. Or in other words, the alleles of clone RH are also present in material used in the present research (see Table 2 and Figure 3).

Testing of markers

The 100 SNP sequences selected as described above were used in a KASPar SNP genotyping system (KBioscience Ltd. Hoddesdon, UK; http://www.kbioscience.co.uk/lab%20services/SNP%20Genotyping/genotyping_chemistry.html). The markers were tested on a set of 20 diploid potato genotypes (see Figure 4) including the three diploid parents used in the present study and further on the donor of the *Sli* gene, clone 07-1004-1 (see also Example 2 herein above).

Results

All SNP sequences generated polymorphisms in the KASPar SNP genotyping system (see Figure 4), corresponding to a 100% success rate. In total, 100 markers x 20 genotypes were tested, comprising 2000 datapoints. We observed 29 missing data, which is less than 2 %. As control, one genotype was tested twice whereby plant samples from different locations were used (Figure 4, columns 4 and 7). All data were identical. These results illustrate that the KASPar SNP genotyping system is an efficient, reliable and reproducible marker platform for diploid potato. This indicated that The KASPar SNP genotyping system is efficient, reliable and reproducible for diploid potato.

All markers showed a high frequency of occurrence in the diploid potato germplasm used in this experiment (Appendix III). The heterozygote frequency in the germplasm by suing these 100 markers ranged from 26 % to 77%, while the *Sli* gene donor was 100% homozygous as is in accordance with expectations (Phumichai et al. 2005 Genome 48: 977–984).

The number of informative markers between the Sli donor and the three parents used in the present study was 54, 52 and 62 resp. This indicates that the KASPar SNP genotyping system is also informative for diploid potato.

Based on the above, it was concluded that the KASPar SNP genotyping system as described herein and based on the markers as developed herein is efficient, reliable and reproducible and very informative to describe the level of homozygosity / heterezygosity in diploid potato.

Table 5A. Sequences of 100 SNP markers in potato

SNPs					Sequence
SNPID	SNPNum	AlleleY	AlleleX	Sequence	Identifier
PotSNP002	11940065	G	C	ACCTGGTGCAGGCCT [C/G] CGTATTGATACGATT	SEQ.ID NO:1
PotSNP011	11940098	C	T	GACTGTGATTCGAAA [T/C] GTTTACCTATCTCTT	SEQ.ID NO:2
PotSNP021	11940075	C	T	TCGTAGAAATGACAT [T/C] TGGGGTGCTAGAGAT	SEQ.ID NO:3
PotSNP026	11940044	C	T	AGTTTCCAGCTGATG [T/C] TATTCTAGGAGACGA	SEQ.ID NO:4
PotSNP032	11940037	G	A	AGAGGCTCTTGAGGC [A/G] CAGCGAAAAGAGGAA	SEQ.ID NO:5
PotSNp038	11940016	C	T	TGGTGACGTTCATGT [T/C] GGACGTAATGCTTCT	SEQ.ID NO:6
PotSNP043	11940036	C	A	AGTATCTCCGTCCGA [A/C] TCACAGTCCTACTCT	SEQ.ID NO:7
PotSNP045	11940094	G	A	GGTGCTAAGATTCGC [A/G] TTGATGATTCACTAG	SEQ.ID NO:8
PotSNP047	11940054	C	T	CCAACACTGGAAAAA [T/C] TATGGAAGCAGAAAG	SEQ.ID NO:9
PotSNP052	11940097	C	T	GCTAGCATTAGAGCT [T/C] GCTAAGCGACTCAAT	SEQ.ID NO:10
PotSNP055	11940021	G	A	GCCTTGGAGTAAGAG [A/G] TTTTCTGTTTTGGGC	SEQ.ID NO:11
PotSNP057	11940040	C	T	GGATGTTCATCAACG [T/C] CGACTTCGACAAGCT	SEQ.ID NO:12
PotSNP061	11940032	C	T	ACAACCTGCAAGAGC [T/C] TTGCATCTGTTTAGT	SEQ.ID NO:13
PotSNP068	11940023	C	T	TACAATCATGATCCT [T/C] TTGCCTAATTGCCCT	SEQ.ID NO:14
PotSNP072	11940005	G	A	CTGAGCTAGTAGATC [A/G] ATCAAACGGTGCTCC	SEQ.ID NO:15
PotSNP073	11940076	G	T	GACTGTATGGACAGT [T/G] ATTGGGTCAGAGGAC	SEQ.ID NO:16
PotSNP080	11940068	C	T	AAGATGACGTGGAGT [T/C] CGGCACTGCGCCGTT	SEQ.ID NO:17
PotSNP081	11940047	C	T	GCCGCACAAGGTGCT [T/C] GCCGTCGATGTTAGC	SEQ.ID NO:18
PotSNP083	11940033	C	T	AGGTAGTGTTGCAAA [T/C] ACAATTAGAGGGTTG	SEQ.ID NO:19
PotSNP089	11940062	G	A	AAGTACATTTATGAG [A/G] AGATTAACAAGTGTT	SEQ.ID NO:20

PotSNP099	11940092	C	T	ACTGCTGGAACTGAG [T/C] TGAACAATGCTATTA	SEQ.ID NO:21
PotSNP100	11940083	C	T	GAGTGCCGGCAGCGT [T/C] ACAGATCCGACGATG	SEQ.ID NO:22
PotSNP102	11940070	G	A	TATGGCAGAGAAAGA [A/G] ATGGAGTATAGAGTG	SEQ.ID NO:23
PotSNP1026	11940059	G	C	TAAACTCCAACATAT [C/G] AACACAAATTAGTGC	SEQ.ID NO:24
PotSNP1034	11940001	G	A	TTCTACATCCTTCGC [A/G] GGCATTTTTGAGGAG	SEQ.ID NO:25
PotSNP1049	11940051	T	C	TTTGGGGAGGAACAC [C/T] TGTGCTTTAGAACTC	SEQ.ID NO:26
PotSNP1057	11940022	A	C	TGTCACTAAGAAAGC [C/A] CAAGAGGCAATGCAG	SEQ.ID NO:27
PotSNP106	11940010	G	T	GCAAGCTACTTGTTC [T/G] AAAGGACCTGCTACT	SEQ.ID NO:28
PotSNP1072	11940027	G	T	AGCGAGTTTGGATGC [T/G] GAGCTTTTGCAGTTA	SEQ.ID NO:29
PotSNP1083	11940019	C	T	TACAAAGTTCACTGC [T/C] GAGGAGGTCATGCAG	SEQ.ID NO:30
PotSNP1105	11940041	A	C	GATGAGGAAAGGCAC [C/A] GGCGGAGGTGTTGGA	SEQ.ID NO:31
PotSNP1115	11940071	T	C	CACCACCGTCCAAAC [C/T] GGTTTTGCCAATCGC	SEQ.ID NO:32
PotSNP1122	11940060	T	C	GGTCCCTGAGCAAGG [C/T] AATCAACGAATTAGG	SEQ.ID NO:33
PotSNP1139	11940004	G	A	AGTCTCTACAAACCC [A/G] TGGTTTCGATCTTGA	SEQ.ID NO:34
PotSNP116	11940058	G	A	TAATGTGGTCGGCAC[A/G] CTGACGCTTGCTGAT	SEQ.ID NO:35
PotSNP118	11940091	G	A	AGATGCATTCTATGC [A/G] GAACCACAAAGATAT	SEQ.ID NO:36
PotSNP120	11940082	G	A	TTCGTGTTGCACTCA [A/G] CGATTCAGTTGGATG	SEQ.ID NO:37
PotSNP121	11940099	C	T	AGCTTGTGCTAATAG [T/C] TGTGCTGATTTTCTC	SEQ.ID NO:38
PotSNP122	11940078	G	A	TATGCAATCCCAGCC [A/G] ACATATCCGATCCAT	SEQ.ID NO:39
PotSNP124	11940055	G	A	TCATACTTCACATAT [A/G] TCAGAGTGGTCTGAT	SEQ.ID NO:40
PotSNP130	11940050	C	T	AGAGAAAAAAGCTGT [T/C] GTTGTTGATGACGAG	SEQ.ID NO:41
PotSNP134	11940080	C	T	CGCGTATTTCCCTTA [T/C] TATAACTCGTTCTCT	SEQ.ID NO:42
PotSNP138	11940052	C	T	TTACATATGCAATAG [T/C] GGTGGTGATCTTTAT	SEQ.ID NO:43

PotSNP152	11940077	C	T	GAAGTTGTCTTACAT [T/C] GCTCTTGACTATGAA	SEQ.ID NO:44
PotSNP162	11940074	C	T	CTATGATGCATTTAG [T/C] TCATGGAGCAAACTT	SEQ.ID NO:45
PotSNP165	11940009	G	A	AGTTCCTTTTTCTCC [A/G] AGTTCTCAGACTCCG	SEQ.ID NO:46
PotSNP178	11940026	G	T	GAGGAATTGATGCGT [T/G] CTGAACTGGCACAGT	SEQ.ID NO:47
PotSNP182	11940095	G	A	ATACACAGGAAGGGT [A/G] AAGTGCTTCAAATTG	SEQ.ID NO:48
PotSNP184	11940038	G	A	TTTGACCATTCTTTC [A/G] GTCCTAGCTAACCAC	SEQ.ID NO:49
PotSNP185	11940002	C	T	AAGATAAGAAGAAAG [T/C] TAAGAGAACCAAGAT	SEQ.ID NO:50
PotSNP194	11940064	C	T	GGCCCACTTTCCACA [T/C] ATTCAATTTGGTCTC	SEQ.ID NO:51
PotSNP205	11940042	G	A	TCAACTCACCACTGG [A/G] TTTTACTCAAAATCA	SEQ.ID NO:52
PotSNP213	11940096	G	A	TAATATGTTGTGTCT [A/G] TAATTTTTGGGTCAT	SEQ.ID NO:53
PotSNP220	11940013	C	T	CATTAACCAAGGTTG [T/C] ATCGTTCGAGAGATC	SEQ.ID NO:54
PotSNP229	11940056	C	T	TCCTTTGTCATTGTA [T/C] GCCATGGAAGAAGCA	SEQ.ID NO:55
PotSNP392	11940003	T	A	GCTTTGTTTAAGAAC [A/T] GCCAGAAGAAGTTGC	SEQ.ID NO:56
PotSNP401	11940012	G	A	GCCTACGCGGCATGA [A/G] GATGTGGATATTGTT	SEQ.ID NO:57
PotSNP402	11940072	T	C	AAAGCTCAAATTAAA [C/T] GACGATGTTCAGGAA	SEQ.ID NO:58
PotSNP423	11940020	A	G	GGCCAACCAATTCCA [G/A] CGTCTTCTCCCAGAC	SEQ.ID NO:59
PotSNP446	11940081	C	T	AAAACCATTTGGTCT [T/C] GTTGATAATTCTGAG	SEQ.ID NO:60
PotSNP458	11940069	A	G	TACATAGCTCTACAA [G/A] CTTGGAAACATGTCA	SEQ.ID NO:61
PotSNP470	11940043	T	A	GACACAAAATTCATA [A/T] TGGCTGATACTTTGT	SEQ.ID NO:62
PotSNP543	11940049	T	A	GCAACTTGTCGTCGG [A/T] AAACTCGACTAGATT	SEQ.ID NO:63
PotSNP567	11940014	A	C	CTAGAAACAAAATCA [C/A] AATATAAGTTACATA	SEQ.ID NO:64
PotSNP569	11940028	G	A	GTTGTTTGCTTTGTT [A/G] TGCTGACTTATTGTA	SEQ.ID NO:65
PotSNP573	11940034	C	T	ATGCTTCTTGGTTGC [T/C] GTTTGTTTAGGCTCT	SEQ.ID NO:66
PotSNP580	11940087	T	C	AAATATGTTGTTCCA [C/T] TAGAAAAAAGAGTAA	SEQ.ID NO:67

PotSNP586	11940088	C	T	GTGAATACTGGTATC [T/C] TTCCAATTGAAGTCA	SEQ.ID NO:68
PotSNP587	11940067	C	T	AATATTCTTGTAGTA [T/C] ATATTGTCTAAAATC	SEQ.ID NO:69
PotSNP607	11940084	T	C	CAACGATATCGAGAA [C/T] TATTGTTCTAAGGGA	SEQ.ID NO:70
PotSNP626	11940045	G	A	GCTGAAAGTAAAGAC [A/G] GTGTTCAACATCTTG	SEQ.ID NO:71
PotSNP645	11940007	T	G	CATGCAATTGTAATA [G/T] TATCAATGTTTGTTG	SEQ.ID NO:72
PotSNP652	11940024	T	C	TGCTCAAGCTGCCAA [C/T] GCTAATCCGCTTTAC	SEQ.ID NO:73
PotSNP700	11940061	G	A	CAAGATATATGCAGC [A/G] GAGTCCCTTCGAGCA	SEQ.ID NO:74
PotSNP702	11940039	A	T	TTATGGTTATGGTGG [T/A] GGAGGTTGGATTAAT	SEQ.ID NO:75
PotSNP712	11940057	C	G	GGGAGGGTGAAAATA[G/C] TCGAAAACGGGCAAC	SEQ.ID NO:76
PotSNP713	11940073	G	A	GATTGTTATCCTTCC [A/G] CCACCTCCAACAGAT	SEQ.ID NO:77
PotSNP731	11940035	A	G	CAACTTCCAGTCGTG [G/A] GAGTGGGGTTAACGG	SEQ.ID NO:78
PotSNP753	11940046	G	A	ATGGTGTCTCCAAGG [A/G] ACTATTACAGTCACT	SEQ.ID NO:79
PotSNP759	11940090	C	T	AACATCGATAGGACT [T/C] TGAAGGCCGCGGATG	SEQ.ID NO:80
PotSNP766	11940089	T	C	CTGCCACTGCGGCTG [C/T] AGGTTCTGCAGCTGC	SEQ.ID NO:81
PotSNP775	11940079	G	A	TGAAATGATTGGATT [A/G] TGACCCATAGTTTAG	SEQ.ID NO:82
PotSNP796	11940025	C	A	ATCTCTAAGTTGGGG [A/C] AACCCATAATTTCTA	SEQ.ID NO:83
PotSNP827	11940053	T	A	CGCTTGGGAAGAACC [A/T] TTCGGTCCAGTTTTG	SEQ.ID NO:84
PotSNP834	11940006	T	C	CCAATTCCGACGGCC [C/T] TCGTCGTTTCACCGC	SEQ.ID NO:85
PotSNP853	11940066	A	C	GGTCGACCATTGGGG[C/A] CCCGTAGAGGGTATA	SEQ.ID NO:86
PotSNP866	11940048	G	T	AAGGCGAATTCCGGT [T/G] CCCGAAATGGATCTC	SEQ.ID NO:87
PotSNP893	11940011	A	G	TCATTACATCAAAAT [G/A] TTTTATCAATGCCCA	SEQ.ID NO:88
PotSNP908	11940100	T	A	TGATCCATTTGGTCT [A/T] GACCAGTTCTTGACA	SEQ.ID NO:89
PotSNP910	11940017	T	A	TGCTGTGCTCCTTCT [A/T] TTCCTCATCTCTCAC	SEQ.ID NO:90
PotSNP928	11940030	A	T	TCATTCTCACTCTCC [T/A] GCTGAACAAGCCATT	SEQ.ID NO:91
PotSNP947	11940015	T	G	AAAAGACAAGAATCT [G/T] GTTCAGATTCTCCAG	SEQ.ID NO:92

PotSNP948	11940063	T	A	TGGGGAAAACTTAAT [A/T] TGACAGCCAAGGCGT	SEQ.ID NO:93
PotSNP960	11940031	C	T	GGTTTTGTGGACTGC [T/C] AACACTGAAAGATAC	SEQ.ID NO:94
PotSNP964	11940029	A	T	TTATTTCAATGAAGC [T/A] TCTGGTGGACGTTAT	SEQ.ID NO:95
PotSNP982	11940085	C	T	TCTGTGGTTTTTTCA [T/C] CACCTCCATCTTCAA	SEQ.ID NO:96
PotSNP983	11940086	G	A	TAAGATTAGTTCTAA [A/G] CTTAGCGCAGAAGAC	SEQ.ID NO:97
PotSNP985	11940018	A	G	GAGCTCTATAAGTGC [G/A] CCTGTTACTCCACCT	SEQ.ID NO:98
PotSNP987	11940008	C	A	GGGCTATGGTTGGTT [A/C] GGAAAAAAGGTGCAA	SEQ.ID NO:99
PotSNP996	11940093	C	T	GGATATTGGGTTCGT [T/C] CCATGATTCGTGGCA	SEQ.ID NO:100

Table 5B: List of PotSNPmarkers and their specific annotation for position on the SH and RH map of Figure 3.

Marker	Annotation	Marker	Annotation	Marker	Annotation
PotSNP072	1.1	PotSNP573	5.1	PotSNP102	9.1
PotSNP834	1.2	PotSNP184	5.2	PotSNP587	9.2
PotSNP645	1.3	PotSNP702	5.3	PotSNP1115	9.3
PotSNP1034	1.4	PotSNP731	5.4	PotSNP402	9.4
PotSNP987	1.5	PotSNP043	5.5	PotSNP713	9.5
PotSNP185	1.6	PotSNP057	5.6	PotSNP080	9.6
PotSNP392	1.7	PotSNP1105	5.7	PotSNP458	9.7
PotSNP165	1.8	PotSNP032	5.8	PotSNP162	9.8
PotSNP1139	1.9	PotSNP205	6.1	PotSNP021	10.1
PotSNP038	2.1	PotSNP753	6.2	PotSNP073	10.2
PotSNP910	2.2	PotSNP470	6.3	PotSNP152	10.3
PotSNP106	2.3	PotSNP026	6.4	PotSNP122	10.4
PotSNP893	2.4	PotSNP626	6.5	PotSNP775	10.5
PotSNP401	2.5	PotSNP081	6.6	PotSNP134	10.6
PotSNP220	2.6	PotSNP866	6.7	PotSNP446	10.7
PotSNP567	2.7	PotSNP124	7.1	PotSNP120	10.8
PotSNP947	2.8	PotSNP047	7.2	PotSNP586	11.1
PotSNP055	3.1	PotSNP543	7.3	PotSNP100	11.2
PotSNP985	3.2	PotSNP130	7.4	PotSNP607	11.3
PotSNP1083	3.3	PotSNP1049	7.5	PotSNP766	11.4
PotSNP423	3.4	PotSNP229	7.6	PotSNP759	11.5
PotSNP1057	3.5	PotSNP712	7.7	PotSNP118	11.6
PotSNP068	3.6	PotSNP138	7.8	PotSNP982	11.7
PotSNP652	3.7	PotSNP827	7.9	PotSNP983	11.8
PotSNP796	3.8	PotSNP1026	8.1	PotSNP580	11.9
PotSNP178	4.1	PotSNP1122	8.2	PotSNP099	12.1

PotSNP1072	4.2	PotSNP700	8.3	PotSNP011	12.2
PotSNP960	4.3	PotSNP853	8.4	PotSNP996	12.3
PotSNP061	4.4	PotSNP089	8.5	PotSNP045	12.4
PotSNP569	4.5	PotSNP948	8.6	PotSNP121	12.5
PotSNP083	4.6	PotSNP194	8.7	PotSNP908	12.6
PotSNP964	4.7	PotSNP116	8.8	PotSNP182	12.7
PotSNP928	4.8	PotSNP002	8.9	PotSNP213	12.8
				PotSNP052	12.9

Table 6. Diploid potato genotypes used for SNP marker development and testing

Nr.	Abbr.	code	Short description	reference
1	H	07-1004-1	*Sli* gene donor	07-1004-1 is a progeny of the *Sli*-donor F1-1 as described in Phumichai et al. (2005) Genome Vol. 48:977-984, Phumichai and Hosaka (2006) Euphytica 149:251–258 and Phumichai et al. (2006) Euphytica 148: 227–234.
2	D1	IVP97-079-9	Parent 1 used in present research, early, long, Y (yellow flesh), Qcook	Examples 1, 2 and 4 herein; available from Ronald Hutten, Laboratory for Plant Breeding, Wageningen University.
3	D2	IVPAA-096-18	Parent 2 used in present research, early, Y, Qcook	Examples 1, 2 and 4 herein; available from Ronald Hutten, Laboratory for Plant Breeding, Wageningen University.
4	D3	SH83-92-488	Parent 3 used in present research, *R3*, *H1*, *Gpa2*, *RXadg*, Y	Examples 2 and 4 herein; available from Ronald Hutten, Laboratory for Plant Breeding, Wageningen University.
5	D4	parent C	Parent of the CxE population	Anithakumari A.M. et al. (2010) Molecular Breeding 26(1):65-75
6	D5	parent E	Parent of the CxE population	Anithakumari A.M. et al. (2010) Molecular Breeding 26(1):65-75
7	D3	SH83-92-488	Independent duplo of nr 3	Van Os, H. et al. (2006) Genetics 173(2):1075-1087
8	D6	RH89-039-16	Used for genome sequencing	Van Os, H. et al. (2006) Genetics 173(2):1075-1087; available from Ronald Hutten, Laboratory for Plant Breeding, Wageningen University
9	D7	3778-16	*Grp1*, early, long (shape), *Ro1*	Rouppe van der Voort et al. (1997) Mol Gen Genetics 255(4):438-447
10	D8	IVP92-057-3	early, long, Y, Qfry	Used for marker development in Example 3
11	D9	IVP98-082-14	Long, Y, Qfry, *H1*, Qcook	Used for marker development in Example 3
12	D10	IVP02-089-5	Early, long, Y, *H1*, Qcook, Zep (orange flesh)	Used for marker development in Example 3
13	D11	IVP05-113-1	Early, y(white flesh)	Used for marker development in Example 3
14	D12	IVP05-122-24	Qstarch, Y	Used for marker development in Example 3
15	D13	IVP06-142-12	Wild species hybrid: Phyt avl	Used for marker development in Example 3
16	D14	IVP06-145-2	Wild species hybrid: Phyt rch	Used for marker development in Example 3

17	D15	IVP06-148-13	Round (shape), Qcook, Qfry	Used for marker development in Example 3
18	D16	IVP06-149-12	Early, round, Zep, Y, spectacled, Qcook, blue anthocyans	Used for marker development in Example 3
19	D17	IVP06-155-9	Wild species hybrid: Phyt tar	Used for marker development in Example 3
20	D18	IVP06-161-16	Early, Phyt vnt1, round, Y, *H1*	Used for marker development in Example 3
21	D19	IVPAA-134-16	Early, round, y (white flesh)	Used for marker development in Example 3

Early: early flowering (short cycle); Qcook: good cooking quality; Qfry: good frying quality; Phyt indicates *Phytophthera* resistance genes

Example 4. Development of homozygous diploid potato plants and generation of heterozygous F1 hybrid progeny

Introduction

The objectives of this Example were to develop homozygous diploid potato lines that are self-compatible (SC) and have good agronomic performance. A breeding programme to combine self-compatibility with good agronomic performance is described above in Example 2 above. The aim of the current experiment is to prove that homozygous diploid self-compatible potato genotypes can be generated by inbreeding and that diploid potato plants containing contrasting homozygous loci scattered over the potato genome can be crossed (are intercrossable) to generate heterozygous F1 hybrid progeny.

Materials and methods

A self-compatible diploid homozygous potato genotype, that harbours the *Sli*-gene as described in the Examples above was crossed with two potato clones, designated D1 and D2 (see Examples 1 and 2) The F1 progenies were selfed and two F2 progenies were generated from two random SC F1 plants. Random self-compatible plants of these two F2 populations were selfed and per F2 population three F3 populations were tested for level of homozygosity and for segregation of SNP markers.

The 6 F2 plants were tested for ploidy level (by fluorescent quantitation of DNA) and were all found to be diploid.

A set of 36 markers was used on 265 individual F3 plants by applying the KASPar SNP genotyping system (see Example 3) covering all 24 chromosome arms of potato (see Figure 5).

Results

Subsets of 35 to 56 plants from the six F3 populations, in total 265 plants, were tested with at least 24 informative markers per parent pair. In total 36 markers were used (see Figure 5). For position of markers on the potato genome see Example 3, above. The *Sli* donor was 100% homozygous for these markers tested, while the D1 and D2 parents contained 20 and 19 loci resp. that were heterozygous out of the 24 informative markers tested per parent pair, while the other five and six loci resp. contained homozygous alleles originating from the from the donor parent (D1 or D2).

Six homozygous F3 plants

As expected, the F3 populations segregated for markers that were heterozygous in the F2 and not for markers that were fixed in the F2 (Figure 5). The mean heterozygote level ranged from 84 to 95 %, while that of individual plants ranged from 71 to 100% (Table 7).

Table 7. Homozygote frequencies (%) of six F3 populations

F3 population	Donor parent	mean	range
126	D1	85	74 – 96
126B	D1	88	74 – 100
127	D1	84	71 – 96
121	D2	85	71 - 96
122	D2	89	79 -100
123	D2	94	88 - 100

Three F3 populations of the D1 x H and of the H x D2 crosses were analysed for frequency of homozygous loci based on the data in Figure 5 as shown in Table 9.

These frequencies are expected giving the allele frequencies of the parents. There were six plants from three populations that were already 100 % homozygous. These harboured mainly homozygous loci with *Sli* donor alleles. This is expected as the *Sli* donor harbours homozygous alleles, while the other parents contained one common allele with the *Sli* donor per heterozygous marker. So, *Sli*–donor derived alleles were abundant in all offspring populations. These six homozygous F3 plants, designated 122-34, 123-6, 123-20,

123-23, 123-24 and 126B-17, contained two, five, four, three, five and seven loci resp. with homozygous alleles from the donor parent. So, already in two selfed generations 100 % homozygosity was reached for 24 marker loci representing 24 chromosome arms.

No absolute lethal allele

It is often stated that repeated selfings in diploid potato will only result in weak plants due to inbreeding depression. This should be due to numerous deleterious alleles that reduce plant fitness, when these alleles are homozygously present. Therefore, we tested the segregation of 36 markers in the six F3 populations mentioned (see Figure 5).

These markers, that were chosen because they are informative for the parent pairs used in this study, were also tested for the frequencies in 19 diploid potato genotypes, that were chosen as donors for the breeding programme (see Example 3, Figure 4 and Table 9). If lethal alleles are present, then loci with homozygous alleles should be absent. Indeed, seven markers (designated PotSNP165, PotSNP205, PotSNP753, PotSNP 238, PotSNP 700, PotSNP 100 and PotSNP 607) did not show homozygous alleles that were different from the *Sli*-donor alleles in this diploid germplasm. Most of these markers were already fixed in the F2, most of them for *Sli* donor alleles as these were more abundant in the parents, but also examples were observed whereby the F3 population was fixed for the allele of the non-*Sli* parent (PotSNP753 in populations 126B and 127). Three of these seven markers (PotSNP205, PotSNP753 and PotSNP100) segregated in the F3 populations, whereby the PotSNP753 segregated normally and PotSNP205 and PotSNP100 showed distorted segregation. These may represent deleterious alleles. Only one plant with homozygous PotSNP100 donor alleles was identified. Without wishing to be bound by theory, it is believed that this may represent a very deleterious allele. As a single plant has been identified with a homozygous allele of the donor parent, this does not provide full proof for an absolute lethal allele.

F3 x F3 crosses

The 265 F3 plants tested were raised in an unheated greenhouse in the summer of 2010. In addition about 600 additional F3 plants were raised in the field with clay soil (see also Example 2). All these plants were from unselected F2 plants grown in a winter-nursery in The Netherlands (see Example 2). Crosses were made by applying pollen from greenhouse and field grown self-compatible plants on stigma of greenhouse grown self-compatible F3 plants. Only crosses were made between F3 plants with different great-grandparents (F3 plants of populations 121 - 123 crossed with 126 – 127). The greenhouse plants designated 126-24, 126-31, 126B-2 and 127-21 were successfully crossed as female

self-compatible parent with a self-compatible F3 plant from population 123 as male parent. The female F3 plants were more than 80% homozygous, while the F3 plant in the field was not tested but the population mean homozygosity level was 94% with a range from 88 to 100%.

The parents of the F3 x F3 crosses were compared for loci that are already fixed in the F3 populations (see Table 8). Ten homozygous loci on nine chromosomes were identified that were at least contrasting between one of the four female and the one male parent used for the F3 x F3 crosses. As a consequence, the F1 hybrid progeny will be at least heterozygous for those contrasting loci. As each individual parent pair contained at least five contrasting loci, each individual progeny plant will harbour at least five contrasting chromosome regions.

Table 8. Contrasting fixed loci between the parents of the F3 x F3 crosses.

		chr	126-24	126-31	126B-2	127-21
Homozygosity level	%		86	80	88	88
Contrasting homozygous loci with F3 population 123	PotSNP392	1	x			
	PotSNP038	2	x	x		x
	PotSNP985	3			x	x
	PotSNP1105	5	x	x	x	x
	PotSNP753	6			x	
	PotSNP124	7				x
	PotSNP162	9	x	x	x	
	PotSNP021	10			x	x
	PotSNP120	10	x	x	x	x
	PotSNP213	12	x	x		
Total #	10	9	6	5	6	6

The codes 126-24, 126-31, 126B-2 and 127-21 refer to individual F3 plants that were used as females in crosses with one field grown plant of the F3 population designated "123" (see above). The homozygosity level was calculated as the percentage of homozygous loci over all loci tested (Figure 5). The "x" refers to homozygous markers that with contrasting alleles between the four female plants and the one plant of population 123.

Conclusions

It was found that after two generations of selfings homozygous diploid potato plants were obtained comprising alleles from both parents. Not a single marker out of 36 markers tested provided proof for a lethal allele. Furthermore, plants with more than 84% homozygous loci can be successfully crossed to obtain hybrid plants with at least five

heterozygous chromosome regions. This means that hybrid seeds that are essentially uniform and that represent true potato seed have now been produced.

Table 9. Allele frequencies of genome wide SNP markers in diploid germplasm and F3 populations.

SNP marker	chrom	allele frequencies											
code	no	diploid germplasm				F3 from H X D1				F3 from H x D2			
		HH**	HD	DD	HD	HH	HD	DD	HD	HH	HD	DD	HD
		#*	#	#	%***	#	#	#	%	#	#	#	%
PotSNP834	1	4	9	5	50	47	79	17	55	122	0	0	0
PotSNP392	1	5	11	3	58	9	32	14	58	122	0	0	0
PotSNP165	*1*	*13*	*5*	*0*	*28*	*142*	*0*	*0*	*0*	n.i.			
PotSNP038	2	2	12	5	63	15	48	80	34	122	0	0	0
PotSNP947	2	8	9	1	50	118	13	12	9	122	0	0	0
PotSNP985	3	3	8	6	47	62	17	63	12	18	46	58	38
PotSNP796	3	2	12	5	63	142	0	0	0	122	0	0	0
PotSNP960	4	6	10	3	53	59	61	20	44	n.i.			
PotSNP569	4	13	2	4	11	n.i.				121	0	0	0
PotSNP928	4	5	13	1	68	141	0	0	0	88	19	15	16
PotSNP184	5	2	7	10	37	61	59	23	41	122	0	0	0
PotSNP1105	5	2	12	5	63	140	0	0	0	19	40	62	33
PotSNP205	*6*	*13*	*6*	*0*	*32*	n.i.				*91*	*27*	*3*	*22*
PotSNP753	*6*	*9*	*10*	*0*	*53*	*28*	*20*	*91*	*14*	n.i.			
PotSNP626	6	9	8	2	42	n.i.				122	0	0	0
PotSNP081	6	12	6	1	32	142	0	0	0	n.i.			
PotSNP124	7	8	9	2	47	85	0	55	0	n.i.			
PotSNP229	7	7	11	1	58	56	0	0	0	15	37	69	31
PotSNP138	*7*	*9*	*10*	*0*	*53*	*55*	*0*	*0*	*0*	*121*	*0*	*0*	*0*
PotSNP1026	8	10	6	3	32	n.i.				80	0	42	0
PotSNP700	*8*	*9*	*9*	*0*	*50*	*140*	*0*	*0*	*0*	*34*	*0*	*0*	*0*
PotSNP116	8	7	9	3	47	140	0	0	0	n.i.			
PotSNP102	9	3	12	2	71	141	0	0	0	122	0	0	0
PotSNP458	9	11	7	1	37	n.i.				122	0	0	0
PotSNP162	9	14	4	1	21	11	31	98	22	n.i.			
PotSNP021	10	0	10	9	53	33	55	53	39	35	0	0	0
PotSNP152	10	0	8	11	42	141	0	0	0	82	23	17	19
PotSNP120	10	9	9	1	47	n.i.				48	23	51	19
PotSNP100	*11*	*14*	*5*	*0*	*26*	*53*	*89*	*1*	*62*	n.i.			
PotSNP607	*11*	*14*	*5*	*0*	*26*	n.i.				*122*	*0*	*0*	*0*
PotSNP580	11	2	5	12	26	142	0	0	0	43	53	24	44
PotSNP099	12	1	10	8	53	56	0	0	0	120	0	0	0
PotSNP996	12	7	8	4	42	142	0	0	0	n.i.			
PotSNP182	12	6	10	3	53	n.i.				56	43	21	36
PotSNP213	12	9	8	1	44	22	38	80	27	n.i.			
PotSNP052	12	4	8	7	42	142	0	0	0	89	18	14	15

#: number of plants; HH: homozygous *Sli*-donor locus; DD: homozygous donor locus; HD: heterozygous locus; % is percentage heterozygous plants, low numbers indicate high level of homozygosity in F3.

Example 5. Breeding scheme for a commercial potato variety

Starting from F3 materials obtained from crosses between an *Sli* donor and another diploid potato as described in Examples 2 and 4 above, a breeding scheme for producing commercial potato varieties is set out herein below. This scheme consists of a number of consecutive steps, which, in combination with the teaching as described herein, will allow the skilled person to arrive at a diploid or tetraploid hybrid potato variety that is as good as or preferable better then existing commercial hybrids and that can be multiplied much faster than conventional bred varieties within five years.

1. Definition of the product variety

Based on the invention as described herein, the production of a novel potato variety based on the production of commercial hybrids is not a matter of chance, but a directed design process, starting with the articulation of the product profile of the variety. Herein, the desired product profile (target product profile) for a specific market (chips potatoes, starch industry, consumption potato must be defined. In addition, evaluation methods for the relevant traits in the product profile must be defined. Preferably, the presence of relevant traits is assessed by diagnostic markers. Such markers are well known in the art of conventional potato breeding.

2. Field trials

Depending on the season at which the development process is started, a suitable trial field is selected. When starting in September or October, it is preferred that a suitable trial field located on the southern hemisphere is selected. Field trials may start with at least 2, preferably more F3 populations, selected from at least one, but preferably more crosses of a diploid *Sli* donor and another diploid potato genotype serving as a donor for an agronomical trait, preferably the trait of vigour, which F3 populations represent the diploid, fertile, self-compatible, essentially homozygous and vigorous potato lines of the invention as exemplified in Example 2. In addition to these F3 populations, F1 progenies are taken from at least two, preferably more selected F2 plants (as exemplified in Example 2 herein above) crossed with at least two, preferably more diploid potato genotypes that are different from the grandparents of the selected F2 plants. At least 50, preferably more

individual plants of these F3 and F1 populations are then grown on the selected potato field and are selfed.

3. Evaluations and selection

During the growth in the field, the frequency of self-compatible plants that carry berries within a period of 2-6 weeks following the onset of flowering is evaluated, and at least two, preferably more F3 and F1 populations with at least 10%, preferably more self-compatible plants are selected. Further, during the growing season, the agronomic characteristics as defined in the product profile of the self-compatible plants in the selected populations is evaluated. Leaf samples of all self-compatible plants of the selected populations are collected for DNA marker testing.

At the end of the growing season the tuber quality and quantity of the self-compatible plants in the selected populations is evaluated, and the DNA marker genotypes is assessed by using diagnostic and random markers. Based on this evaluation and assessment, at least the five, preferably more, self-compatible plants of each population are selected based on: (i) the combination of traits that is closest to the target product profile; (ii) the highest level of homozygosity, and (iii) with the maximum number of contrasting homozygous loci. The selected and selfed F3 and F1 plants provide F4 and F2 progenies, respectively, in the form of seeds.

4. Tuber and seeds harvest and shipment

The tubers of the selected F3 and F1 plants as well as the F2 and F4 seeds in the berries of these selected plants are harvested at a period of at least at six weeks, preferably more, after flowering. The F4 seeds are collected for further field trials involving testing of the general combining abilities between F4 plants grown thereform, and the F2 seeds provide additional gene pools for breeding and are collected for further field trials. Depending on the season and in order to safe time, the seeds may suitably be shipped to the northern hemisphere for growing in a greenhouse, provided that all phytosanitary regulations of the countries involved are met.

5. Tuber storage and evaluation

The tubers of the selected F1 and F3 plants are preferably stored in the region of cultivation to avoid undesired spread of pathogens and pests, and these stored tubers are evaluated for some relevant intrinsic tuber traits of the stored tubers as defined in the product profile.

6. Tuber propagation and evaluations after 1 year

The tubers of the selected F1 and F3 plants are grown the next season, preferably in the same region as the original potato field to avoid spread of unwanted pathogens or pest. At least two, preferably more potato tubers are used per plot and preferably one preferably more replicates per clone are used. At least one, preferably more relevant commercial cultivars are included as controls. Side-rows with commercial cultivars are preferably included to avoid side effects. The relevant plant characteristics as defined in the product profile (e.g. cooking, frying, chips making, starch content, etc) are evaluated. Further evaluated are the tuber yield per plot as well as the intrinsic relevant tuber characteristics as defined in the product profile. Such evaluations are used for family characterisation and progeny selections. These trials are repeated each year, preferably at locations in both hemispheres to continuously select the ancestor properties of plants, grown in the field. At later stages, when hybrids are obtained that are tested for commercial introductions, the evaluation trials are extended to at least two, preferably more, replicates at plot sizes of at least one, preferably more m^2 and at two, preferably more, locations preferably in but not restricted to the target markets.

7. Testcrosses with F4 seeds and field trials

Soon after the collection of the F4 progeny seeds of the selected selfed F3 plants, at least fifty, preferably more, of these seeds are sown. If needed, this sowing may occur in a heated greenhouse. The seedlings are preferably transplanted in small pots about 1 month after sowing and then transferred to the field in a suitable location some weeks thereafter. Evaluations and selections as described above are continued and at least two, preferably more selected F4 plants per population are testcrossed with at least two, preferably more, other selected F4 progeny plants of two, preferably more selected parents with maximum number of contrasting loci to produce test hybrids (F4 x F4 -> hybrid). Additionally, at least two, preferably more selected F4 plants per population may be crossed with at least one, preferably more diploid genotypes with desirable characteristics or traits that are missing in the selected plants to enhance the genetic diversity of the breeding germplasm. Further, at least two, preferably more selected F4 plants per population may be selfed to produce F5 seeds. Still further, plant parts of at least two, preferably more selected F4 plants per population may be sterilised, *in vitro* propagated and treated with suitable chemicals (colchicine / oryzaline) to produce tetraploid F4 genotypes, and testcrosses are made with tetraploid F4 plants between the same parents as described above. In addition, similarly F2 progeny seeds are sown, transplanted and evaluated, crossed and selfed but not test/crossed

The above breeding scheme is repeated each year whereby an incremental increase in plant traits as defined in the product profile is achieved. The evaluations of the tubers from testcrosses are instrumental to determine the general combining abilities of parents. General combining ability is defined herein as the average performance of a line in a series of crosses. For each market at least two, preferably more elite parent lines are achieved that will render commercial hybrid seeds. It is expected that in five years a diploid or tetraploid hybrid variety is generated that is as good as or preferable better then existing commercial hybrids and that can be multiplied much faster than conventional bred varieties.

Electronic Acknowledgement Receipt

EFS ID:	37491111
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First Named Inventor/Applicant Name:	Willem Hendrik Lindhout
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Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Corrected Filing Receipt	VONL004USC1_Request_for_Corrected_Filing_Receipt.pdf	634451 0a9100320e9bb374ab0d8f31ae926ba13c6d43c3	no	15

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3	Drawings-only black and white line drawings	VONL004USC1_Replacement_Drawings.pdf	2334353 0017dd7e1df174582d704d92514c1e71e58e6ffc	no	41
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4	Applicant Arguments/Remarks Made in an Amendment	VONL004USC1_Marked_Substitute_Specification.pdf	582412 950486c681712a757f401069ef72e67e9a4b93dc	no	66
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If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 16/541,071

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	75		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	330		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	380		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	14 minus 20 =	*	x 50 =	0.00	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 minus 3 =	*	x 230 =	0.00			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0.00			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0.00			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	785		TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)		RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/541,071	08/14/2019	Willem Hendrik Lindhout	VONL:004USC1

CONFIRMATION NO. 4348

125771
DENTONS US LLP
P.O. Box 061080
Chicago, IL 60606

INFORMAL NOTICE

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Date Mailed: 10/22/2019

INFORMATIONAL NOTICE TO APPLICANT

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

The item(s) indicated below are also required and should be submitted with any reply to this notice to avoid further processing delays.

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):
  Willem Hendrik Lindhout
  Teunis Philippus Schotte
  Richard Gerardus Franciscus Visser
  Herman Johannes van Eck
  Ronaldus Cornelis Bernardus Hutten

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
16/541,071	08/14/2019	1662	865	VONL:004USC1	14	3

CONFIRMATION NO. 4348

UPDATED FILING RECEIPT

125771
DENTONS US LLP
P.O. Box 061080
Chicago, IL 60606

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Date Mailed: 10/22/2019

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

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Inventor(s)

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Power of Attorney: The patent practitioners associated with Customer Number 125771

Domestic Priority data as claimed by applicant

This application is a CON of 13/504,156 07/10/2012
which is a 371 of PCT/NL10/50716 10/26/2010
which claims benefit of 61/254,980 10/26/2009

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)
EUROPEAN PATENT OFFICE (EPO) 09013474.3 10/26/2009 No Access Code Provided

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The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 16/541,071**

Projected Publication Date: 01/30/2020

Non-Publication Request: No

Early Publication Request: No
**** SMALL ENTITY ****
Title

Hybrid Seed Potato Breeding

Preliminary Class

800

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

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Oct 22, 2019 03:39:10 AM

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Application	Document	Mailroom Date	Attorney Docket No.
16541071	M327	10/22/2019	VONL:004USC1
	APP.FILE.REC	10/22/2019	VONL:004USC1

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/541,071	08/14/2019	Willem Hendrik Lindhout	VONL:004USC1	4348

125771 7590 10/23/2019

DENTONS US LLP
P.O. Box 061080
Chicago, IL 60606

EXAMINER	
ART UNIT	PAPER NUMBER
1663	

NOTIFICATION DATE	DELIVERY MODE
10/23/2019	ELECTRONIC

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The time period for reply, if any, is set in the attached communication.

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Decision Granting Request for Prioritized Examination (Track I)	**Application No.** 16/541,071	**Applicant(s)** Lindhout et al.	
	Examiner BRIAN W BROWN	**Art Unit** OPET	**AIA (FITF) Status** No

1. THE REQUEST FILED 14 August 2019 IS **GRANTED** .

The above-identified application has met the requirements for prioritized examination

A. ☑ for an original nonprovisional application (Track I).
B. ☐ for an application undergoing continued examination (RCE).

2. **The above-identified application will undergo prioritized examination**. The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:

A. filing a **petition for extension of time** to extend the time period for filing a reply;

B. filing an **amendment to amend the application to contain more than four independent claims, more than thirty total claims** , or a multiple dependent claim;

C. filing a **request for continued examination** ;

D. filing a notice of appeal;

E. filing a request for suspension of action;

F. mailing of a notice of allowance;

G. mailing of a final Office action;

H. completion of examination as defined in 37 CFR 41.102; or

I. abandonment of the application.

Telephone inquiries with regard to this decision should be directed to BRIAN BROWN at (571)272-5338.

In his/her absence, calls may be directed to Petition Help Desk at (571) 272-3282.

/BRIAN W BROWN/ Petitions Examiner, OPET	

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Bescheinigung	Certificate	Attestation
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Patentanmeldung Nr.	**Patent application No.**	**Demande de brevet n°**
	09013474.3 / EP09013474	

The organization code and number of your priority application, to be used for filing abroad under the Paris Convention, is EP09013474.

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le President de l'Office européen des brevets
p.o.

R.C. van Dijk

Aardevo Exhibit 1010
Page 0380

Anmeldung Nr:
Application no.: 09013474.3
Demande no :

Anmeldetag:
Date of filing: 26.10.09
Date de dépôt :

Anmelder / Applicant(s) / Demandeur(s):

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Burg. Arriensweg 32
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Bezeichnung der Erfindung / Title of the invention / Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se référer à la description.)

Hybrid seed potato breeding

In Anspruch genommene Priorität(en) / Priority(Priorities) claimed / Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen / State/Date/File no. / Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation / International Patent Classification / Classification internationale de brevets:

A01H

Am Anmeldetag benannte Vertragstaaten / Contracting States designated at date of filing / Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO SE SI SK SM TR

Hybrid Seed Potato Breeding

FIELD OF THE INVENTION

The invention relates to the field of agriculture, in particular to new plants and processes for obtaining them. The invention also relates to methods for improving the genetic constitution of crop plants, and to plants thus obtained. The invention further relates to a method for producing hybrid seeds and to hybrid seeds thus produced and to methods of producing crops using the hybrid seeds of the invention.

BACKGROUND OF THE INVENTION

The potato (*Solanum tuberosum* L.) is an integral part of the global food system. It is the world's number one non-grain food commodity, with production reaching a record 325 million tonnes in 2007.

Unlike other major field crops, potatoes are reproduced vegetatively from other potatoes. Therefore, a part of each year's crop - ranging from 5 to 15 percent, depending on the quality of the harvested tubers - is set aside for re-use in the next planting season. Most farmers in developing countries select and store their own seed tubers. In developed countries, farmers are more likely to purchase disease-free "certified seed" from dedicated suppliers.

Seed potatoes are more difficult to produce and supply than grain or pulse seed. A seed:harvest ratio of 1:20 for potatoes is considered good, compared to 1:400 for maize or 1:10.000 for tomato. One hectare may therefore require two tonnes of seed material to maximize the yield of harvestable products, compared to 18 kg for maize. In order to break dormancy, seed potatoes should be stored for several weeks before they can be planted. The right conditions during storage such as amount of light, temperature and humidity are crucial to ensure good seed quality.

In addition to the poor seed:harvest ratio, seed potatoes attract and transport pests. These include (amongst others) late blight, Andean potato weevils, nematodes, tuber moths and viruses. The latter are transmitted in the field by aphids and then carried from generation to generation in the seed. Such a virus infection can decrease yields by up to 20 percent. Seed

potatoes have high transportation costs because of the great distances between the major seed production areas and the major consumer production areas and the relatively high weight of individual seed potato tubers.

To feed the growing world population now and in future the potato industry has to keep growing to meet the needs of the consuming public. Substantial research and development efforts are devoted to the modernization of planting and harvesting of fields and processing of potatoes, and to the development of economically advantageous potato varieties. Through crossbreeding of potatoes, researchers hope to obtain potatoes with the desirable characteristics of good processing, both for fresh consumption as well as for industrial purposes, high soluble solids content, high yield, resistance to diseases and pests and adaptability to various growing areas and conditions.

The research leading to potato varieties which combine the advantageous characteristics referred to above is largely empirical. This research requires large investments of time, manpower, and money. The development of a potato cultivar can often take up to eight years or more followed by at least five years of propagation to get sufficient quantities for commercial usage. Breeding begins with careful selection of superior parents to incorporate the most important characteristics into the progeny. Since all desired traits usually do not appear in one progeny, breeding is a continuous process of selecting the best recombinants, combining favourable traits of the ancestors.

The arduous task of producing a new potato variety is best understood when understanding the genetics of potato. The commercial potato has a tetraploid genome. Diploid tubers are generally too small for important commercial applications. In addition, the tetraploid genome is extremely heterozygous, often harbouring multiple alleles per locus. It is believed that self-incompatibility, which is primarily experienced at diploid level, and inbreeding depression are responsible for the maintenance of the high genetic variability found in potato and that overdominance of heterozygous alleles (heterosis) results in vigorous plants. In a typical potato offspring obtained from a cross between two unrelated parental lines deleterious alleles may therefore contribute to either a reduced fitness in case of homozygosity or an increased vigour in case of heterozygosity. It is clear that a breeder needs large populations to maximise

the chance of finding individuals that carry a relatively high number of heterozygotic loci and a low number of homozygotic loci, while also exhibiting beneficial combinations of agronomically desirable traits.

Present potato breeding techniques rely on the controlled crossing of parental clones which themselves are the result of a comprehensive pre-breeding development during which amonst others special techniques such as chromosome doubling, embryo rescue, and somatic fusion are applied in order to introduce the beneficial characteristics of for instance wild and primitive *Solanum* species into these clones. The parental material that is found suitable for further breeding after a phenotypic selection procedure is then mutually crossed and the resulting non-uniform hybrid seeds are sown in large numbers in greenhouses. From tens of thousands of individual F1 seedlings tubers are harvested and retained for the next year's planting. The next year a single "seed" tuber from each resulting seedling is planted in the field. Extreme caution must be taken to avoid the introduction of viruses and diseases since the material is only clonally (vegetatively) expanded before it is sold to individual customers years later. After the second year, samples of tubers are taken for density measurements and preliminary fry tests to determine the suitability of the tubers for commercial use. A multitude of tubers of plants which have survived the selection process to this point are then planted in the third year for a more comprehensive series of fry tests and density determinations. At the fourth-year stage of development, a diminishing number of surviving selections is grown in ever expanding numbers and plants thereof are subjected to field trials in several stages to determine their adaptability to different growing conditions. Eventually, the varieties having superior agronomical qualities are transferred to other farms and the "seed" (in the form of tubers) is increased to commercial scale. Since one "seed" tuber may generate between 6 and 20 harvested tubers this up-scaling process may take years before sufficient "seed" is produced. Generally, by this time, eight or more years of planting, harvesting and testing have been invested in attempting to develop a new and improved potato cultivar.

To reduce inbreeding depression a breeder may introduce new genes from a genetically more remote parent such as from wild and primitive species with ploidy levels ranging from dip-

loid to hexaploid. However, when two genetically unrelated potato plants are crossed, the level of heterozygosity may be increased but simultaneously more deleterious genes are also introduced. As a consequence, a breeder will typically make additional crosses with more commercial germplasm to enrich the population for favourable alleles. All together such a multiple crossing breeding programme may take dozens of years as the selection of the favourable genotypes in each generation may already take five years. Therefore, potato breeding is currently a predominantly empirical exercise, strongly characterised by trial and error.

Potatoes and their related wild species (tuber-bearing *Solanum* species) are mostly outbreeding because self fertilisation is hampered by a gametophytic self-incompatibility system. Self-incompatibility (SI) is a general name for several genetic mechanisms in angiosperms, which prevent self-fertilization and inbreeding. In plants with SI, when a pollen grain produced in a plant reaches a stigma of the same plant or another plant with a similar genotype, the process of pollen germination, pollen tube growth, ovule fertilization, and embryo development is halted at one of its stages, and consequently no seeds are produced. Self-incompatibility is not found in tetraploid potatoes.

The provision of such self-compatible clones may facilitate the generation of selfed progenies of potato, and hence the production of (highly) homozygous potato lines. This could provide a great opportunity to the development of homozygous elite breeding lines in potato. However, to date, the development of homozygous elite lines with genetically fixed agronomically desirable traits, that would enable the production of genetically uniform hybrid potato seed, has been unsuccessful.

The provision of homozygous elite lines is hampered by unknown causes. Selfing of an occasionally encountered self-compatible clone results in a slower decrease of heterozygosity than theoretically expected. The slower decline of heterozygosity may be the result of unintentional but unavoidable selection during selfing for the heterozygous plants in the offspring that exhibit higher vigour, fertility and seed germination. It is implied that, as the heterozygosity is reduced by selfing, the fertility and vigour are also reduced and the plants may become weak and completely sterile. The situation is perhaps worsened by the establishment of homozygous configurations of recessive

deleterious genes. This phenomenon which is generally referred to as inbreeding depression has greatly hampered the development for homozygous potato lines and hence the production of uniform hybrid potato seed.

It is an objective of the present the invention to provide means and methods for the production of elite breeding lines of potato and for the production of uniform hybrid potato seed.

SUMMARY OF THE INVENTION

The present invention further provides in a first aspect a method for producing a genetically homozygous true potato seed (TPS), comprising:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, self-compatible and essentially homozygous potato line,

(b) providing a second potato plant, wherein said second potato plant is a plant of a second potato line, preferably of a fertile, self-compatible and essentially homozygous potato line, said second potato plant carrying a chromosome with an allele for a agronomically desirable trait that is to be expressed in an offspring generation, and

(c) cross pollinating said first and second potato plant such that one of said parent plants produces berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed carrying a chromosome with said allele.

This method results in the production of an essentially homozygous, vigorous and self-compatible breeding line. The provision of such a breeding line is a crucial step in the provision of genetically homozygous true potato seed.

The provision of a first and second breeding line can then be used in the production of the TPS when both the first and second breeding line comprise agronomically desirable characteristics.

Thus, the invention also provides as an embodiment of a method as described above a method comprising the steps of:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, self compatible and essentially homozygous potato line carrying chromosomes with alleles for agronomically desirable traits that are to be expressed in

an offspring generations,

(b) providing a second potato plant, wherein said second potato plant is a plant of a second diploid, self compatible and essentially homozygous potato line carrying chromosomes with alleles for agronomically desirable traits that are to be expressed in an offspring generations and that may be different form the traits in the said first plant, and

(c) cross pollinating said first and second potato plant such that one of said parent plants produces berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed carrying combinations of chromosomes with said alleles.

Thus, in an alternative embodiment the plant of the first potato line may carry chromosomes with alleles for agronomically desirable traits that are to be expressed in an offspring generations, in which case the agronomically desirable trait in the plant of the second potato line may be different from the traits in the said first plant.

In a preferred embodiment of a method of the invention, the agronomically desirable trait is selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, rooting ability, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, growth rate, tuber development defects (e.g. misshapen or damaged tubers), tuber yield, tuber size, tuber skin color, eye depth, tuber shape, tuber flesh color, tuber taste, tuber storage ability, tuber dormancy period, tuber shelf life, resistance to tuber dehydration, tuber starch content, tuber dry matter content, tuber cooking quality, tuber frying quality, tuber chip making quality, tuber uniformity and cold sweetening resistance.

In a preferred embodiment of a method of the invention said first potato plant is a plant of potato line AGVD1, AGVD2 or AGVD3, representative seeds of said lines having been deposited on 23 October 2009 with the NCIMB, Aberdeen, Scotland under breeder's reference AGVD1, AGVD2 or AGVD3, respectively. The deposits received the accessions numbers NCIMB 41663 (for AGVD1), NCIMB 41664 (for AGVD2) and NCIMB 41665 (for AGVD3).

In another preferred embodiment of a method of the invention said second potato plant is a plant of a second diploid, self-compatible and essentially homozygous potato line.

In another preferred embodiment of a method of the invention, said second diploid, self-compatible and essentially homozygous potato line is obtained in an additional step of (e) selfing said hybrid offspring potato plant or backcrossing said hybrid offspring potato plant to a potato plant of said line of said first potato plant, thereby providing further offspring plants carrying a chromosome with said allele and wherein said offspring plants are increasingly homozygous following each backcrossing or selfing step.

More preferably said step (e) optionally comprises one selfing, and may further comprise from 2 to 7 or more backcrosses using a plant of line AGVD1, AGVD2 or AGVD3 as the recurrent parent and further comprises the step of testing offspring plants for the homozygous or heterozygous presence of alleles for the trait, optionally using genetic markers for said allele, optionally followed by one or two selfings, so as to provide a homozygous plant that is genetically essentially equal to individual plants of line AGVD1, AGVD2 or AGVD3, respectively, but in which alleles responsible for the trait are heterozygously, preferably homozygously present.

In another aspect, the present invention provides a seed of potato line AGVD1, AGVD2 or AGVD3, representative seeds of said lines having been deposited on 23 October 2009 with the NCIMB, Aberdeen, Scotland under breeders reference AGVD1 (NCIMB 41663), AGVD2 (NCIMB 41664) and AGVD3 (NCIMB 41665), respectively.

In another aspect, the present invention provides a potato plant, or part thereof, produced by growing the above seed. In a preferred embodiment, the plant is a plant of line AGVD3.

A plant of the present invention is a vigorous, diploid, self compatible and essentially homozygous potato plant, preferably comprising 1, more preferably, 2, 3, 4, 5, 6, 7 or more agronomically desirable traits selected from the group of agronomically desirable described above.

In yet another aspect, the present invention provides pollen or an ovule of the plant produced by growing the deposited seed described here above.

In still another aspect, the present invention provides a potato plant having all of the physiological and morphological characteristics of the plant produced by growing the deposited seed described here above.

In yet another aspect, the present invention provides a tissue culture of the plant produced by growing the deposited seed described here above.

In yet another aspect, the present invention provides a potato plant regenerated from the tissue culture described above, wherein said regenerated potato plant has all the physiological and morphological characteristics of a potato plant produced by growing the deposited seed described here above.

In another aspect, the present invention provides a method for producing a hybrid potato seed wherein the method comprises crossing a first potato plant with a second potato plant and harvesting the resultant hybrid potato seed, wherein said first or second parent potato plant or both said first and second potato plant is the potato plant produced by growing the deposited seed described here above.

In another aspect, the present invention provides a method of producing a herbicide resistant, nematode resistant, insect resistant and/or disease resistant potato plant, said method comprising producing a seed according to a method of the invention described above by crossing parental lines that are herbicide resistant, nematode resistant, insect resistant and/or disease resistant and growing said seed a potato plant.

In another aspect, the present invention provides a herbicide resistant, nematode resistant, insect resistant and/or disease resistant potato plant produced by the method of the invention as described above.

In yet another aspect, the present invention provides a method for preventing transmittal of virus infections between potato generations, comprising:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, self-compatible and essentially homozygous potato line,

(b) providing a second potato plant, wherein said second potato plant is a plant of a potato line that may have any level of ploidy and/or any level of homozygosity, said second potato plant carrying a chromosome with an allele for a

agronomically desirable trait that is to be expressed in an offspring generation;

(c) cross pollinating said first and second potato plant such that one of said parent plants produces berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed carrying a chromosome with said allele;

(d) growing said hybrid offspring seed into a hybrid offspring potato plant carrying a chromosome with said allele, and

(e1) harvesting tubers from said hybrid offspring potato plant to provide tubers expressing said trait, or

(e2) selfing said hybrid offspring potato plant or backcrossing said hybrid offspring potato plant to a potato plant of said line of said first potato plant, thereby providing further offspring plants carrying a chromosome with said allele and wherein said offspring plants are increasingly homozygous following each backcrossing or selfing step.

In another aspect, the invention provides a method for producing a diploid potato breeding line, comprising:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, self-compatible and essentially homozygous potato line,

(b) providing a second potato plant, wherein said second potato plant is a plant of a potato line that may have any level of ploidy and/or any level of homozygosity, said second potato plant carrying a chromosome with an allele for a agronomically desirable trait that is to be expressed in an offspring generation;

(c) cross pollinating said first and second potato plant such that one of said parent plants produces berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed carrying a chromosome with said alleleand,

(d) growing said hybrid offspring seed into a hybrid offspring potato plant carrying a chromosome with said allele, and

(e) selfing said hybrid offspring potato plant or backcrossing said hybrid offspring potato plant to a potato plant of said line of said first potato plant, and providing further offspring plants carrying a chromosome with said allele wherein

said offspring plants are increasingly homozygous following each backcrossing or selfing step to thereby providing the diploid potato breeding line.Suitable traits in such methods of the invention are indicated above.

In preferred embodiments of methods of the invention said first potato plant is a plant of potato line AGVD1, AGVD2 or AGVD3, representative seeds of said line having been deposited on 23 October 2009 with the NCIMB, Aberdeen, Scotland under breeders reference AGVD1, AGVD2 and AGVD3, respectively.

In other preferred embodiments of methods of the invention said second potato plant is a plant of a second diploid, self-compatible and essentially homozygous potato line. Preferably, said second diploid, self-compatible and essentially homozygous potato line is obtained by performing the method for preventing transmittal of virus infections between potato generations as described above.

In preferred embodiments of methods of the invention, said step of selfing said hybrid offspring potato plant or backcrossing said hybrid offspring potato plant preferably comprises one selfing, and further comprises from 2 to 7 backcrosses using a plant of line AGVD1, AGVD2 or AGVD3 as the recurrent parent and preferably further comprises the step of testing offspring plants for the homozygous or heterozygous presence of alleles for the trait, optionally using genetic markers for said allele, preferably followed by one or two selfings, so as to provide an essentially homozygous plant that is genetically essentially equal to a plant of population AGVD1, AGVD2 or AGVD3, but in which alleles responsible for the trait are heterozygously, preferably homozygously present.

The method for producing a diploid potato breeding line may further comprise the steps of enhancing the breeding value of the diploid potato breeding line by intercrossing, selfing and backcrossing while continuous selecting for agronomically desirable traits. Experimental crosses are done to evaluate general combining abilities.

In another aspect, the present invention provides a diploid potato breeding line, more preferably an elite line, obtainable, preferably obtained, by the method of the present invention.

In another aspect, the present invention provides a hybrid potato cultivar obtainable, preferably obtained, by cross-

ing a first and second diploid potato breeding line according the present invention.

DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic presentation of a development scheme for the development of hybrid potato cultivars using the methods and plants of the present invention, wherein the various steps in obtaining commercial potato hybrids are indicated. 0, Potato germplasm: The origin comprises in principle all potato genotypes within the cultivated potato species (*Solanum tuberosum*) and related crossable species; 1, Self-compatible diploid potato: Cultivated potato (*Solanum tuberosum*) is usually self incompatible. Self-compatibility is introduced from related species (see for instance Hermsen, J.G.Th. 1978, Euphytica 27, 1-11.); 2, Homozygous fertile self-compatible diploid potato: Homozygous diploids can be obtained by doubling haploids (Uijtewaal et al., 1987, Theor. Appl. Genet. 73, 751-78) by anther culture (Jacobsen and Sopory, 1977, Theor. Appl. Genet. 52, 119-123), by ovule culture or by repeated selfings (Phumichai & Hosaka, 2006, Euphytica 149, 251-258). The example of the latter is illustrated herein; 3, Vigorous fertile homozygous self-compatible diploid potato: The vigour is enhanced by crossing with other diploid potato, selfing and backcrossing while selecting for higher vigour; 4, Diploid potato breeding Lines: Agronomically desirable traits are introduced by crossing with other diploid potato, selfing and backcrossing while selecting for these traits; 5, Elite diploid potato breeding Lines: The breeding value is further enhanced by intercrossing, selfing and backcrossing while continuous selecting for agronomically desirable traits. Experimental crosses are done to evaluate general combining abilities; 6 Potato hybrid cultivars: Good combining breeding lines are crossed. The seeds obtained are hybrid true potato seeds that are uniform.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

The term "potato" is used herein to refer to material that is essentially of species *Solanum tuberosum*, but may include introgression segments of other tuber bearing *Solanum* species such as *Solanum chacoense, Solanum phureja, Solanum andigena, Solanum demissum*.

The term "potato plant" is used herein to refer to a seedling or mature plant as grown from cell culture or seed. Persons of ordinary skill in the art will recognize that when the term "potato plant" is used in the context of the present invention, this also includes derivative varieties that retain the essential distinguishing characteristics of for instance potato varieties that are subject of the present invention, such as a Single Gene Converted plant of that variety or a transgenic derivative having one or more value-added genes incorporated therein (such as herbicide or pest resistance). Backcrossing methods can be used with the present invention to improve or introduce a characteristic into the variety.

The term "crossing" as used herein refers to the fertilization of female plants (or gametes) by male plants (or gametes). The term "gamete" refers to the haploid reproductive cell (egg or sperm) produced in plants by mitosis from a gametophyte and involved in sexual reproduction, during which two gametes of opposite sex fuse to form a diploid zygote. The term generally includes reference to a pollen (including the sperm cell) and an ovule (including the ovum). "Crossing" therefore generally refers to the fertilization of ovules of one individual with pollen from another individual, whereas "selfing" refers to the fertilization of ovules of an individual with pollen from the same individual. Crossing is widely used in plant breeding and results in a mix of genomic information between the two plants crossed one chromosome from the mother and one chromosome from the father. This will result in a new combination of genetically inherited traits. Usually, the progeny of a crossing is designated as: "F1". If the F1 is not uniform (segregates) it is usually designated as "F1 population". "Selfing" of a homozygous plant will usually result in a genetic identical plant since there is no genetic variation. "Selfing" of an F1 will result in an offspring that segregates for all traits that have heterozygotic loci in the F1. Such offspring is designated: "F2" or "F2 population"

When referring to "crossing" in the context of achieving the introgression of a genomic region or segment, the skilled person will understand that in order to achieve the introgression of only a part of a chromosome of one plant into the chromosome of another plant, it is required that random portions of the genomes of both parental lines will be recombined during

the cross due to the occurrence of crossing-over events in the production of the gametes in the parent lines. Therefore, the genomes of both parents must be combined in a single cell by a cross, where after the production of gametes from said cell and their fusion in fertilization will result in an introgression event.

As used herein, the terms "introgressing", "introgress" and "introgressed" refer to both a natural and artificial process whereby individual genes or entire chromosomes are moved from one individual, species, variety or cultivar into the genome of another individual, species, variety or cultivar, by crossing those individuals, species, varieties or cultivars. In plant breeding, the process usually involves selfing or backcrossing to the recurrent parent to provide for an increasingly homozygous plant having essentially the characteristics of the recurrent parent in addition to the introgressed gene or trait.

The term "introgression" refers to the result of an introgression event.

The term "backcross" refers to the result of a "backcrossing" process wherein the plant resulting from a cross between two parental lines is (repeatedly) crossed with one of its parental lines, wherein the parental line used in the backcross is referred to as the recurrent parent. Repeated backcrossing results in replacement of genome fragments of the donor parent with those of the recurrent. The offspring of a backcross is designated "BCx" or "BCx population", where "x" stands for the number of backcrosses.

The term "backcrossing" as used herein refers to the repeated crossing of a hybrid progeny back to the recurrent parents. The parental potato plant which contributes the gene for the desired characteristic is termed the nonrecurrent or donor parent. This terminology refers to the fact that the nonrecurrent parent is used one time in the backcross protocol and therefore does not recur. The parental potato plant to which the gene or genes from the nonrecurrent parent are transferred is known as the recurrent parent as it is used for several rounds in the backcrossing protocol. In a typical backcross protocol, the original variety of interest (recurrent parent) is crossed to a second variety (nonrecurrent parent) that carries the single gene of interest to be transferred. The resulting progeny from this cross are then crossed again to the recurrent parent

and the process is repeated until a potato plant is obtained wherein essentially all of the desired morphological and physiological characteristics of the recurrent parent are recovered in the converted plant, in addition to the single gene or a limited number of genes transferred from the nonrecurrent parent.

The selection of a suitable recurrent parent is an important step for a successful backcrossing procedure. The goal of a backcross protocol is to alter or substitute a single trait or characteristic in the original variety. To accomplish this, a single gene of the recurrent variety is modified, substituted or supplemented with the desired gene from the nonrecurrent parent, while retaining essentially all of the rest of the desired genes, and therefore the desired physiological and morphological constitution of the original variety. The choice of the particular nonrecurrent parent will depend on the purpose of the backcross. One of the major purposes is to add some commercially desirable, agronomically important traits to the recurrent parent. The exact backcrossing protocol will depend on the characteristic or trait being altered or added to determine an appropriate testing protocol. Although backcrossing methods are simplified when the characteristic being transferred is a dominant allele, a recessive allele may also be transferred. In this instance, it may be necessary to introduce a test of the progeny to determine if the desired characteristic has been successfully transferred. Preferably, such genes are monitored by diagnostic molecular markers.

Likewise, transgenes can be introduced into the plant using any of a variety of established recombinant methods well-known to persons skilled in the art.

Many single gene traits have been identified that are not regularly selected for in the development of a new variety but that can be improved by backcrossing and genetic engineering techniques. Single gene traits may or may not be transgenic, examples of these traits include but are not limited to: herbicide resistance; resistance to bacterial, fungal or viral disease; insect resistance; uniformity or increase in concentration of starch and other carbohydrates; enhanced nutritional quality; decrease in tendency of tuber to bruise; and decrease in the rate of starch conversion to sugars.

The term "selfing" refers to the process of self-fertilization wherein an individual is pollinated or fertilized

with its own pollen. Repeated selfing eventually results in homozygous offspring.

A "line", as used herein, refers to a population of plants derived from a single cross, backcross or selfing. The individual offspring plants are not necessarily identical to one another. It is possible that individual offspring plants are not vigorous, fertile or self compatible due to natural variability. However, it is foreseen that suitable plants that are vigorous, fertile and self compatible can be easily identified in a line and used for additional breeding purpose.

As used herein, the term "allele(s)" means any of one or more alternative forms of a gene, all of which alleles relate to at least one trait or characteristic. In a diploid cell or organism, the two copies of a gene occupy corresponding loci on a pair of homologous chromosomes. Each copy may be a distinct allele.

A "gene" is defined herein as a hereditary unit (often indicated by a sequence of DNA) that occupies a specific location on a chromosome and that contains the genetic instruction for a contribution to potential phenotypic characteristics or trait in a plant.

A "locus" is defined herein as the position that a given gene occupies on a chromosome of a given plant species.

As used herein, the term "homozygous" means a genetic condition existing when identical alleles reside at corresponding loci on homologous chromosomes.

The term "essentially homozygous" refers to a level of homozygosity of at least 50%, preferably at least 75%,moe preferably at least 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% homozygosity when testing 100, preferably 1000, more preferably more than 10.000 loci.

Homozygosity levels are average values for the population, and refers preferably to those loci wherein the parent differ.

As used herein, the term "heterozygous" means a genetic condition existing when different alleles reside at corresponding loci on homologous chromosomes.

The term "recombination" or "recombine" refers to the exchange of information between two homologous chromosomes during meiosis. In a "recombinant" plant, DNA that is originally

present on a specific location within the chromosome, e.g. linked to a gene/locus, is exchanged for DNA from another plant (i.e. maternal for paternal or *vice versa*). In order to exchange only the required material, and maintain the valuable original information on the chromosome as much as possible, two flanking crossover or recombination events will usually be required. In a double recombinant this exchange has taken place on both sides of a gene/locus. one way to find such a double recombinant, is to screen a population of F2-plants. This population has to be large, since double recombination occurs in only a limited frequency. Alternatively, double recombinants within a genetic unit can be the result of subsequent backcrossing. The frequency of double recombination is the product of the frequencies of the single recombinants. (E.g. a recombinant in a 10 cM area can be found with a frequency of 10%, double recombinants are found with a frequency of 10% x 10% = 1 %).

As used herein, the term "progeny" means (a) genetic descendant(s) or offspring.

As used herein, the term "population" means a genetically heterogeneous collection of plants sharing a common genetic derivation.

A "recombination event" refers to a mitotic or meiotic crossing-over event, including a GMO event.

As used herein, the term "hybrid" means any offspring of a cross between two genetically unlike individuals, more preferably the term refers to the cross between two (elite or inbred) breeding lines which will not reproduce true to the parent from seed.

The term "segregate", as used herein, refers to the separation of paired alleles during meiosis so that members of each pair of alleles appear in different gametes. The term includes reference to the result of this genetic phenomenon wherein the offspring population of a crossing in which at least one of the parents is heterozygous for an allelic gene is non-uniform with respect to phenotypic trait conferred by said gene.

The term "breeding line", as used herein, refers to a line of a cultivated potato having commercially valuable or agronomically desirable characteristics, as opposed to wild varieties or landraces. The term includes reference to an elite breeding line or elite line, which represents an essentially homozygous, usually inbred, line of plants used to produce commer-

cial F_1 hybrids. An elite breeding line is obtained by breeding and selection for superior agronomic performance comprising a multitude of agronomically desirable traits. An elite plant is any plant from an elite line. Superior agronomic performance refers to a desired combination of agronomically desirable traits as defined herein, wherein it is desirable that the majority, preferably all of the agronomically desirable traits are improved in the elite breeding line as compared to a non-elite breeding line. Elite breeding lines are essentially homozygous and are preferably inbred lines.

The terms "cultivar" and "variety" are used interchangeable herein and denote a plant having a biological status other than a "wild" status, which "wild" status indicates the original non-cultivated, or natural state of a plant or accession.

The term "cultivar" includes, but is not limited to, semi-natural, semi-wild, weedy, traditional cultivar, landrace, breeding material, research material, breeder's line, synthetic population, hybrid, founder stock/base population, inbred line (parent of hybrid cultivar), segregating population, mutant/genetic stock, market class and advanced/improved cultivar. Examples of cultivars include such cultivated varieties as Bintje, Russet Burbank, Eigenheimer, and Nicola.

As used herein, the term "pure inbred" or "inbred" refers to a substantially homozygous plant or plant line obtained by repeated selfings.

As used herein, the term "molecular genomic marker" or short "marker" refers to an indicator that is used in methods for visualizing differences in characteristics of nucleic acid sequences. Examples of such indicators are restriction fragment length polymorphism (RFLP) markers, amplified fragment length polymorphism (AFLP) markers, single nucleotide polymorphisms (SNPs), insertion/deletion (INDEL) mutations, microsatellite markers (SSRs), sequence-characterized amplified regions (SCARs), cleaved amplified polymorphic sequence (CAPS) markers or isozyme markers or combinations of the markers described herein which defines a specific genetic and chromosomal location.

As used herein, the term "plant part" indicates a part of the potato plant, including organelles, single cells and cell tissues such as plant cells that are intact in plants, cell

clumps and tissue cultures from which potato plants can be regenerated. Examples of plant parts include, but are not limited to, single cells and tissues from pollen, ovules, leaves, embryos, roots, root tips, tubers, anthers, flowers, fruits, stems shoots, and seeds; as well as pollen, ovules, leaves, embryos, roots, root tips, anthers, flowers, fruits, stems, shoots, scions, rootstocks, seeds, tubers, protoplasts, calli, and the like.

Unless expressly stated otherwise, the term "seed", as used throughout this specification, refers to the body from which a new plant develops (or kernel in some plants) containing the small embryonic plant enclosed in a seed coat covering, usually together with some stored food. This seed, referred to as botanical or "true" seed is the product of the ripened ovule of gymnosperm and angiosperm plants which occurs after fertilization and some growth within the mother plant.

As used herein the term "vigorous" refers to a plant that within 100 days of cultivation under normal growth conditions that prevail in North Western Europe during the season from May trough to August attains a weight of the foliage and shoots (above-ground plant) expressed in grams of fresh or dry weight which is at least 50%, more preferably at least 60%, 70%, 80%, 90% or more of the diploid breeding clone RH89-039-16, which clone is available for reference purpose from Dr. Herman van Eck, curator of the Laboratory of Plant Breeding, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen. The term vigour may therefore also refer to a tuber yield expressed in grams of fresh or dry weight which at harvest is at least 50%, more preferably at least 60%, 70%, 80%, 90% or more of the diploid breeding clone RH89-039-16.

The terms "fertile" and "fertility" broadly refer to the capacity to reproduce, i.e. to conceive and to produce offspring, preferably fertile offspring. Thus, the term preferably refers to individuals that can be crossed to produce fertile offspring.

The term "fertile offspring" or "fertile seed" is defined herein as seed capable of growing into a flower-producing potato plant wherein flowers are male and female fertile. Thus, the term preferably refers to a plant or seed that when grown into a plant is capable of producing offspring as male and as female parent by virtue of the presence of fertile ovules and

fertile pollen (i,e, both male and female flowers are fertile).

The term "fertile plant" is defined herein as a plant capable of producing fertile seed carrying berries. Preferably said berries each carry at least 5, more preferably at least 15-20, even more preferably at least 50 fertile seed, still more preferably between 50 and 500 seeds. Alternatively, a plant is said to be fertile when the number of seeds produced per berry is at least 10%, more preferably at least 30%, 40%, 50%, 60%, 70%, 80%, 90% or more of the number found in berries of the diploid breeding clone RH89-039-16.

The term "self-compatible" refers to capacity of self-pollination, self fertilization and producing fertile progeny. The term "capable of being backcrossed/selfed/crossed and resulting in (self) fertile and self-compatible offspring" refers to the capability of producing seed as a result of backcrossing, selfing or crossing and wherein more than 60%, preferably more than 70%, 80%, 85%, 90% or more of said seed is capable of developing into plants that are (self) fertile and self-compatible.

Description of the preferred embodiments

The present inventors discovered that elite breeding lines for potato breeding can be successfully produced. Crossing of two elite breeding lines provides F1 hybrid seeds, such F1 hybrid seeds, when grown into plants, result in plants of superior agronomic performance. More importantly, all seeds are genetically essentially identical, meaning that with the exception of an exceptional crossover or mutation event, all seeds are uniform.

The present inventors have thus discovered a method that allows the provision of elite potato breeding lines, which, when crossed mutually, provide uniform F1 hybrid potato seeds that, when grown into plants, result in plants having superior agronomic performance. The elite potato breeding lines may have complementary traits and as a result of their homozygosity the plants grown from the F1 hybrid potato seeds are uniform, but when these F1 plants are subsequently self-pollinated this will result in F2 plants in which the traits segregate.

Thus, in contrast to the currently available true potato seeds (TPS) generated in the conventional way and harvested from berries in which every individual seed is genetically dif-

ferent from every other seed, the seeds obtained from crossing of the elite potato breeding lines in accordance with aspects of the present invention are genetically essentially identical.

The TPS varieties of the prior art are derived from crosses between selected but heterozygous parents and hence variability is expected in plants and tubers. Hybrid potato breeding as proposed herein differs from the conventional production of TPS in that the latter are the result of open pollination or crossing between two genetically non-homozygous breeding lines resulting in non-uniform cultivars and the present is the result of the crossing of two pure breeding lines, resulting in uniform cultivars.

Novel potato plants

Parental breeding lines

The present inventors have generated potato plants that are essentially homozygous, diploid, vigorous, fertile and self-compatible and having fixed within their genome nucleic acid sequences encoding genes that confer to the plant an agronomically desirable trait. The nucleic acid sequences are the result of introgressions from potato germplasm having agronomically desirable traits. Conventionally, homozygous plants can in principle be obtained in as little as two generations by doubling of monohaploids generated from unfertilised generative cells such as egg or pollen cells by methods known as doubled haploid technology.

The present inventors used the strategy of repeated selfing, backcrossing and crossing of potato germplasm of a self-compatible and self incompatible potato lines. The homozygous plants of the present invention were obtained by continuous selfing and selection for fertile offspring. Generally, around 7 to 8 selfing cycles will result in essentially homozygous plants, but the skilled artisan will understand that more generations of selfing may also be used. Also fewer generations, such as six generations may be used to provide essentially homozygous plants of the present invention.

The essentially homozygous potato plants of the present invention have as an advantage that they can be crossed with other essentially homozygous potato plants whereby the hybrid offspring of said cross is essentially genetically uniform and will not segregate.

Thus the present invention now provides essentially homozygous, diploid, vigorous, fertile, and self-compatible potato plants having agronomically desirable traits. The novel potato plants of the invention are capable of:

- being backcrossed to an essentially homozygous recurrent parent and resulting in (self) fertile and self-compatible offspring;
- being selfed (self-fertilization of 1 offspring individual) and resulting in (self) fertile and self-compatible offspring;
- being mutually crossed (fertilization between 2 offspring individuals) and resulting in (self) fertile and self-compatible offspring.

Plants according to the present invention include the line with breeders reference AGVD1, representative seeds of said line having been deposited on 23 October 2009 with the NCIMB, Aberdeen, Scotland under breeders reference AGVD1, which plant represents an F2 obtained by crossing a plant of parent P1, which is an essentially homozygous, diploid, fertile and self-compatible but non-vigorous potato line with a plant of parent P2, which is an elite diploid breeding clone that is essentially non-homozygous, and is not self-compatible, but which has the desirable agronomic trait of yellow flesh color and exhibits good cooking quality in that the flesh is not greying after cooking, and selfing the resulting F1 offspring plants. It is estimated that AGVD1 is about 50% homozygous, meaning that about 50% of the loci for which the parents differ are homozygous.

Plants according to the present invention also include the line with breeders reference AGVD2, representative seeds of said line having been deposited on 23 October 2009 with the NCIMB, Aberdeen, Scotland under breeders reference AGVD2, which line represents an (F1 x F1) line (or pseudo F2) obtained by crossing a plant of parent P1 as indicated above with a plant of parent P2 as indicated above, and mutually crossing individual F1 offspring plants, that share the P1 parent but have different individuals of the P2 parent. It is estimated that AGVD2 is about 25% homozygous for the loci for which the parents differ.

Plants according to the present invention also include the line with breeders reference AGVD3, representative seeds of said line having been deposited on 23 October 2009 with the

NCIMB, Aberdeen, Scotland under breeders reference AGVD3, which line represents a BC1 population obtained by crossing a plant of parent P1 as indicated above with a plant of parent P2 as indicated above, and backcrossing the resulting F1 offspring plants with the P1 parent. It is estimated that AGVD3 is about 75% homozygous for the loci for which the parents differ.

A plant of the present invention can very suitably be used as a recurrent parent wherein additional agronomically desirable traits are introgressed by crossing, or introduced by other means such as by transgenic means. Once a number of agronomically desirable traits have been stacked into an essentially homozygous, vigorous, diploid, fertile and self-compatible plant according to the invention, that plant can be developed into and used as an elite breeding line for the production of commercial F_1 hybrid potato seed. Likewise, a second elite breeding line can be provided that can serve as the parent of opposite sex so that both plants can be crossed to produce the commercial F_1 seed of a novel potato variety.

The breeding of uniform hybrid seed can be achieved when i) an initial introgression crossing can be made between a plant of the invention and a plant that acts as donor for an agronomically desirable trait, that ii) a backcross can be produced between the offspring of that initial crossing and the plant of the invention, and that iii) the offspring thereof can be selfed. Potato lines AGVD1, AGVD2 and AGVD3 exhibit these characteristics and individual plants of these lines can be used in additional introgression crossings, backcrossings and selfings to arrive at the elite breeding lines suitable for use as parents for commercial F1 hybrid seed that can be grown into hybrid potato plant of a new potato variety. A most preferred plant according to the present invention is a plant of line AGVD2 or an offspring plant thereof having all of the physiological and morphological characteristics of the plant of line AGVD2.

Hybrids

The hybrid plants of the invention are produced by a method of the invention as exemplified in Figure 1. The hybrid plants of the invention are produced by crossing two essentially homozygous elite potato breeding lines comprising among them a number of agronomically desirable traits selected from the group

consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, rooting ability, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, growth rate, tuber development defects (e.g. misshapen or damaged tubers), tuber yield, tuber size, tuber skin color, eye depth, tuber shape, tuber flesh color, tuber taste, tuber storage ability, tuber dormancy period, tuber shelf life, resistance to tuber dehydration, tuber starch content, tuber dry matter content, tuber cooking quality, tuber frying quality, tuber chip making quality, tuber uniformity and cold sweetening resistance.

Novel method of potato seed breeding

The provision of the novel potato plants now provides for the first time the attainment of the method of the invention.

Essentially, the novel method of producing hybrid potato cultivars provides for the possibility to produce a new potato variety within about 5 years.

The present invention now provides a method for producing a genetically homogenous or uniform true potato seed (TPS). This method is *inter alia* useful in a method of preventing transmittal of virus infections between potato generations, but also for overcoming many of the other problems associated with classical potato breeding, "seed" potato production and transport and cultivation. Moreover, the present parental material for production of hybrid seed can be maintained in the form of seed, and not in the form of vegetative material as is required by traditional TPS production.

The present invention provides in one aspect a method for producing a hybrid potato cultivar comprising the use of the deposited plant material provided herein as a starting material.

Alternatively, other essentially homozygous, diploid, fertile, vigorous and self-compatible plant lines different from the deposits referred to herein may be developed and the production of a hybrid potato cultivar can commence from that early stage level by using the teaching provided herein since the present inventors have demonstrated that this route, although laborious, is successful. Such an alternative breeding material is

also vigorous, essentially homozygous, diploid, fertile and self-compatible.

As a first step, such an early stage development may comprise the provision of a plant of a first cultivated potato species (*Solanum tuberosum*) or a related crossable species (generally a tuber bearing *Solanum* species).

Next, said plant is developed into a line of self-compatible diploid potato plants, preferably a *Solanum tuberosum*. Diploid potato is usually self incompatible. Self-compatibility may suitably be introduced from related species (see for instance Hermsen, J.G.Th. 1978, Euphytica 27, 1-11).

The resulting line of self-compatible diploid potato plants is then developed into a homozygous fertile self-compatible diploid potato line. Homozygous diploids can be obtained by doubling haploids (Uijtewaal et al., 1987, Theor. Appl. Genet. 73, 751-78) by anther culture (Jacobsen and Sopory, 1977, Theor. Appl. Genet. 52, 119-123), by ovule culture or by repeated selfings (Phumichai & Hosaka, 2006, Euphytica 149, 251-258). The latter method was used for producing the homozygous fertile self-compatible diploid potato line that formed the basis of the vigorous plants as described herein.

Next, the homozygous fertile self-compatible diploid potato line is developed into a vigorous fertile homozygous self-compatible diploid potato line. The vigour may be enhanced by crossing with other diploid potato plants, selfing and backcrossing while selecting for higher vigour.

Next, the vigorous, fertile, homozygous, self-compatible, diploid potato line is developed into a diploid potato breeding line. Most preferably, at least two separate vigorous fertile homozygous self-compatible diploid potato lines are produced and at least two distinct diploid potato breeding lines are developed. Such breeding lines comprise a high number of agronomically desirable traits. Agronomically desirable traits are introduced by crossing with other diploid potato plants, selfing and backcrossing while selecting for these traits. This breeding program may comprise testing for disease, such as whether the shoots or tubers are free of disease, or whether they are resistant for disease.

Next, the diploid potato breeding lines are developed into elite diploid potato breeding lines. This is accomplished by enhancing the breeding value by intercrossing, selfing and

backcrossing while continuously selecting for agronomically desirable traits. At this stage experimental crosses are performed to evaluate general combining abilities. The diploid elite lines can now also be developed into tetraploid (4x) elite lines by methods known per sé, such as by chemical treatment with e.g. colchicine, or by "spontaneous" doubling of the genome using tissue culture.

Finally, potato hybrid cultivars are produced from these elite diploid or tetreploid potato breeding lines by crossing of good combining breeding lines. The seeds obtained are hybrid true potato seeds that are uniform.

The method of the invention for producing a potato hybrid cultivar comprises the steps of producing a diploid potato breeding line. Said method comprises the steps of:

(a) providing a first potato plant, which first potato plant is a plant of a diploid, self-compatible and essentially homozygous potato line, preferably a plant of a vigorous fertile homozygous self-compatible diploid potato line, more preferably a diploid potato breeding line;

(b) providing a second potato plant of a potato line that may have any level of ploidy, preferably diploid, and/or any level of homozygosity wherein said second potato plant serves as a donor for an agronomically desirable trait which trait is to be introgressed into said essentially homozygous line of said first potato plant or at least in an offspring plant resulting from a crossing between said first and second plant, wherein said trait is selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, rooting ability, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, growth rate, tuber development defects (e.g. misshapen or damaged tubers), tuber yield, tuber size, tuber skin color, eye depth, tuber shape, tuber flesh color, tuber taste, tuber storage ability, tuber dormancy period, tuber shelf life, resistance to tuber dehydration, tuber starch content, tuber dry matter content, tuber cooking quality, tuber frying quality, tuber chip making quality, tuber uniformity and cold sweetening resistance.

Following the provision of these two plants, the method then

comprises the step of:

(c) cross-pollinating said first and second potato plant such that one of said parent plants produces seed-carrying fruits (berries) and collecting said seeds from said fruits to provide a hybrid offspring seed.

Seeds are then grown to plants and the plants that comprise the agronomically desirable trait from the donor plant are selected, either directly or following additional selfing and backcrossing steps, as is well known in the art of plant breeding. In these methods markers may be used to assist in the breeding process.

The hybrid offspring seed is preferably grown into a plant that is subsequently backcrossed a plant to the recurrent parent line

The following steps are not essential to either of the above methods. However, these additional steps may either provide for the production of consumption tubers by growing the seeds of the invention and/or may provide for the production of new homozygous, self-compatible, diploid elite breeding lines that can serve as parents for the inclusion of yet further traits into an offspring generation. The plants of the invention are fertile and vigorous. These additional benefits are attained by the following further steps of:

(d) growing said offspring seed into a hybrid offspring potato plant, and

(e1) harvesting tubers from said hybrid offspring potato plant to provide tubers carrying said trait, or

(e2) selfing said hybrid offspring potato plant or backcrossing said hybrid offspring potato plant to a potato plant of said line of said first potato plant to provide further offspring plants carrying said trait and wherein said of offspring plants are increasingly homozygous following each backcrossing or selfing step.

Preferably said second potato plant used in the method described above is a hybrid offspring potato plant as defined in step (d) above and obtained by the method as described above. More preferably, said second potato plant used in the method described above is a hybrid offspring potato plant as defined in step (e2) above and obtained by the method as described above.

Preferably, said step (e2) comprises from 2 to 7 backcrosses using a plant of line AGVD1, AGVD2 or AGVD3 as the re-

current parent and testing offspring plants for the presence of the trait (e.g. in the form of the homozygous or heterozygous presence of alleles responsible for the trait), followed by one or two (or more) selfings, to provide an essentially homozygous plant that is genetically equal to a plant of population AGVD1, AGVD2 or AGVD3, but in which alleles responsible for the trait are heterozygously, preferably homozygously present. This plant has at least one additional allele not available in population AGVD1, AGVD2 or AGVD3.

The above method now provides a breeding process for the production of homozygous breeding lines or elite breeding lines based on the background of population AGVD1, AGVD2 or AGVD3, to which background successively additional alleles are added, thereby providing elite breeding lines wherein desirable traits are stacked.

The method of the present invention now for the first time provides for a method for preventing transmittal of virus infections between potato generations while at the same time also solving many of the other problems of the prior art related to breeding of new potato varieties and commercial production of potatoes for consumption. This has become possible through the provision of true potato seed, that is, botanical seed, which seed can be grown to a potato plant that produces tubers for consumption, and which seeds are from a cross in which at least one, preferably both of the parents are plants of the same or a different diploid, self-compatible and essentially homozygous potato line. In this way, the transmittal of infection with a virus that is not spread through the gametes and seeds can effectively be prevented.

The present inventors now provide a method for further breeding for homozygous lines that are easy to handle, and which exhibit improved fertility and plant vigour when compared to prior art inbreds, while maintaining the self-compatibility and high level of homozygosity. It will be appreciated that - just as in tomato - a 100% homozygosity is not always needed to provide for an effective breeding program. This is not a problem as long the self-compatibility is maintained.

It has now for the first time been shown that traits can be genetically transferred into the homozygous background of a recurrent parent by repeated backcrossing.

Based on the means and methods provided herein, the

skilled person will be able to stack new traits into the presently provided self-compatible and essentially homozygous lines in order to establish a population of lines that are enriched for agronomically desirable traits and that can serve as novel germplasm for commercial breeding.

CLAIMS

1. A method for producing a uniform hybrid potato seed, comprising:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, self-compatible and essentially homozygous potato line,

(b) providing a second potato plant, wherein said second potato plant is a plant of a second potato line, said second potato plant carrying a chromosome with an allele for a agronomically desirable trait that is to be expressed in an offspring generation, and

(c) cross pollinating said first and second potato plant such that one of said parent plants produces berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed carrying a chromosome with said allele.

2. The method of claim 1, wherein said trait is selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, rooting ability, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, growth rate, tuber development defects (e.g. misshapen or damaged tubers), tuber yield, tuber size, tuber skin color, eye depth, tuber shape, tuber flesh color, tuber taste, tuber storage ability, tuber dormancy period, tuber shelf life, resistance to tuber dehydration, tuber starch content, tuber dry matter content, tuber cooking quality, tuber frying quality, tuber chip making quality, tuber uniformity and cold sweetening resistance.

3. The method of claim 1 or 2, wherein said first potato line is potato line AGVD1, AGVD2 or AGVD3, representative seeds of said lines having been deposited on 23 October 2009 with the NCIMB, Aberdeen, Scotland under breeders references AGVD1, AGVD2 and AGVD3, respectively, or a diploid, self-compatible and essentially homozygous offspring potato plant thereof, preferably an offspring plant comprising an additional agronomically desirable trait.

4. The method of any one of claims 1-3, wherein said second potato plant is a plant of a second diploid, self-compatible and essentially homozygous potato line.

5. The method of claim 4, wherein said second diploid, self-compatible and essentially homozygous potato line is obtained by performing the method of claim 1, further comprising the step of (d) growing said hybrid offspring seed into a hybrid offspring potato plant carrying a chromosome with said allele, and (e) selfing said hybrid offspring potato plant or backcrossing said hybrid offspring potato plant of step (d) to a potato plant of said line of said first potato plant, thereby providing further offspring plants carrying a chromosome with said allele and wherein said offspring plants are increasingly homozygous following each backcrossing or selfing step.

6. The method of claims 5, wherein said step (e) optionally comprises one selfing, and further comprises from 2 to 7 backcrosses using a plant of line AGVD1, AGVD2 or AGVD3 as the recurrent parent and further comprises the step of testing offspring plants for the homozygous or heterozygous presence of alleles for the trait, optionally using genetic markers for said allele, optionally followed by one or two selfings, so as to provide an essentially homozygous plant that is genetically essentially line AGVD1, AGVD2 or AGVD3, respectively, but in which alleles responsible for the trait are heterozygously, preferably essentially homozygously present.

7. A seed of potato line AGVD1, AGVD2 or AGVD3, representative seeds of said line having been deposited on 23 October 2009 with the NCIMB, Aberdeen, Scotland under breeders reference AGVD1, AGVD2 and AGVD3, respectively.

8. A potato plant, or part thereof, produced by growing the seed of claim 7.

9. Pollen or an ovule of the plant of claim 8.

10. A potato plant having all of the physiological and morphological characteristics of the plant of claim 8.

11. A tissue culture of the plant of claim 8.

12. A potato plant regenerated from the tissue culture of claim 11, wherein said regenerated potato plant has all the physiological and morphological characteristics of a potato plant of claim 8.

13. A method for producing a hybrid potato seed wherein the method comprises crossing a first potato plant with a second

potato plant and harvesting the resultant hybrid potato seed, wherein said first or second parent potato plant or both said first and second potato plant is the potato plant of claim 8.

14. A method of producing a herbicide resistant, nematode resistant, insect resistant and/or disease resistant potato plant, said method comprising producing a seed according to a method according to any one of claims 1-6 or 13 by crossing parental lines that are herbicide resistant, nematode resistant, insect resistant and/or disease resistant and growing said seed a potato plant.

15. A herbicide resistant, nematode resistant, insect resistant and/or disease resistant potato plant produced by the method of claim 14.

16. A method for producing a diploid potato breeding line, comprising:

(a) providing a first potato plant, which first potato plant is a plant of a first diploid, self-compatible and essentially homozygous potato line,

(b) providing a second potato plant, wherein said second potato plant is a plant of a potato line that may have any level of ploidy and/or any level of homozygosity, said second potato plant carrying a chromosome with an allele for a agronomically desirable trait that is to be expressed in an offspring generation;

(c) cross pollinating said first and second potato plant such that one of said parent plants produces berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed carrying a chromosome with said alleleand,

(d) growing said hybrid offspring seed into a hybrid offspring potato plant carrying a chromosome with said allele, and

(e) selfing said hybrid offspring potato plant or backcrossing said hybrid offspring potato plant to a potato plant of said line of said first potato plant, and providing further offspring plants carrying a chromosome with said allele wherein said offspring plants are increasingly homozygous following each backcrossing or selfing step to thereby providing the diploid potato breeding line.

17. The method of claim 18, wherein said trait is selected from the group consisting of insect tolerance, nematode

resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora infestans*), herbicide tolerance, rooting ability, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, growth rate, tuber development defects (e.g. misshapen or damaged tubers), tuber yield, tuber size, tuber skin color, eye depth, tuber shape, tuber flesh color, tuber taste, tuber storage ability, tuber dormancy period, tuber shelf life, resistance to tuber dehydration, tuber starch content, tuber dry matter content, tuber cooking quality, tuber frying quality, tuber chip making quality, tuber uniformity and cold sweetening resistance.

18. The method of claim 16 or 17, wherein said first potato plant is a plant of potato line AGVD1, AGVD2 or AGVD3, representative seeds of said line having been deposited on 23 October 2009 with the NCIMB, Aberdeen, Scotland under breeders reference AGVD1, AGVD2 and AGVD3, respectively.

19. The method of any one of claims 16-18, wherein said second potato plant is a plant of a second diploid, self-compatible and essentially homozygous potato line.

20. The method of claim 19, wherein said second diploid, self-compatible and essentially homozygous potato line is obtained in step (e2) by performing the method of claim 1.

21. The method of any one of claims 16-20, wherein said step (e2) optionally comprises one selfing, and further comprises from 2 to 7 backcrosses using a plant of line AGVD1, AGVD2 or AGVD3 as the recurrent parent and further comprises the step of testing offspring plants for the homozygous or heterozygous presence of alleles for the trait, optionally using genetic markers for said allele, optionally followed by one or two selfings, so as to provide a homozygous plant that is genetically essentially equal to a plant of line AGVD1, AGVD2 or AGVD3, respectively, but in which alleles responsible for the trait are heterozygously, preferably homozygously present.

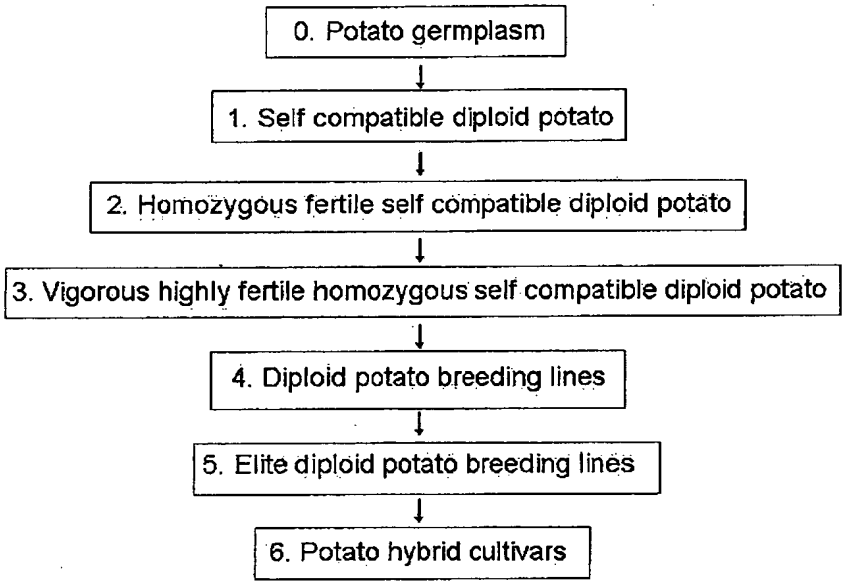
22. A diploid potato breeding line, more preferably an elite line, obtainable by the method of any one of claims 16-21.

23. A hybrid potato cultivar obtainable by crossing a first and second diploid potato breeding line according to claim 22.

ABSTRACT

The present invention relates to a method for producing a genetically homozygous true potato seed (TPS), comprising the steps of (a) providing a first potato plant, which first potato plant is a plant of a first diploid, self-compatible and essentially homozygous potato line, (b) providing a second potato plant, wherein said second potato plant is a plant of a potato line, said second potato plant carrying a chromosome with an allele for a agronomically desirable trait that is to be expressed in an offspring generation, and (c) cross pollinating said first and second potato plant such that one of said parent plants produces berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed carrying a chromosome with said allele. The invention further relates to seeds thus obtained and to potato plants grown from said seed.

Figure 1


0. Potato germplasm
1. Self compatible diploid potato
2. Homozygous fertile self compatible diploid potato
3. Vigorous highly fertile homozygous self compatible diploid potato
4. Diploid potato breeding lines
5. Elite diploid potato breeding lines
6. Potato hybrid cultivars

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16/541,071	08/14/2019	Willem Hendrik Lindhout	VONL:004USC1	4348

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EXAMINER	
BOGGS, RUSSELL T	

ART UNIT	PAPER NUMBER
1663	

NOTIFICATION DATE	DELIVERY MODE
12/05/2019	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

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Office Action Summary	Application No. 16/541,071	Applicant(s) Lindhout et al.	
	Examiner RUSSELL T BOGGS	Art Unit 1663	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 14 August 2019.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 35-48 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 35-48 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☑ Other: See Continuation Sheet.

Continuation of Attachment(s) 4) Other: Request under 37 CFR 1.105 attached to Office action

DETAILED ACTION

Notice of Pre-AIA or AIA Status

The present application is being examined under the pre-AIA first-to-invent provisions.

Status

1. Claims 35-48 as filed on 14 August 2019 are examined herein.

Examiner's Notes

Citations to Applicant's specification are abbreviated herein "Spec."

Claim 43 depends from claim 42 and further limits step (b) but there is no step (b) explicitly in claim 42, only in claim 41. Since claim 42 depends from claim 41 the claim is definite and there is no reason to object to it but nevertheless it is possible that Applicant intended claim 43 to depend directly from claim 41.

Specification

2. The disclosure is objected to because of the following informalities.

The disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code. Applicant is required to delete the embedded hyperlink and/or other form of browser-executable code. *See* MPEP § 608.01. Hyperlinks are found in the specification on page 9 (two), page 51 and page 52.

The specification is objected to because it refers to "Appendix III" on p. 52, l. 20. No such appendix exists.

The specification is also objected to because it does not include the full address of NCIMB in the deposit statement on pages 33-35.

Appropriate correction is requested.

Sequence Rules

3. This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821 (a)(I) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821 through 1.825.

Sequence identifiers are missing from pages 53-55.

Full compliance with the sequence rules is required in response to this Office action. Applicant can either give each sequence present within the tables a unique identifier number, or specify them as a range with respect to an already identified sequence. A complete response to this Office action must include both compliance with the sequence rules and a response to the issues set forth herein. Failure to fully comply with both of these requirements in the time period set forth in this Office action will be held to be non-responsive.

Claim Objections

4. Claims 37 and 39 are objected to because of the following informalities.

Claim 37 is objected to because the limitation "cold sweeting" in the last line of the claim is an obvious typographical error for 'cold sweetening' and should also should be hyphenated. See claims 43 and 47 which use the correct terminology.

Claim 39 is objected to because it requires that the potato line has a certain level of foliage weight. It is hard to imagine that any potato line would fail to meet this limitation and therefore Applicant's intention was probably to refer to an individual plant of said plant line.

Appropriate correction is requested.

Claim Rejections - 35 USC § 112(b)

The following is a quotation of 35 U.S.C. 112(b):

> (B) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. Claims 35 and 41-40 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

Claim 35 recites the limitation "the potato line" in line 1. There is insufficient antecedent basis for this limitation in the claim because the prior occurrence of a line in the claim refers to a *Solanum* species plant line.

Claim 41 recites the limitation "said line" in line 2 of part (e). There is insufficient antecedent basis for this limitation in the claim because the only prior occurrence of "line" in the claim is in the preamble were it refers to line that is being produced by the method steps.

Claim 41 is also rejected because it is internally contradictory. The specification defines "essentially homozygous" as requiring only 25% homozygosity. Therefore there is no requirement that a plant produced by the first backcross be 50% homozygous.

Dependent claims are included in this rejection because none provide limitations obviating this rejection.

Claim Rejections - 35 USC § 112(a) (written description)

The following is a quotation of 35 U.S.C. 112(a):

> (a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), first paragraph:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

6. Claims 35-48 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint

inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention.

Applicant filed claims that contain new matter.

Claims 35-39, 41 and 44-45 recite the limitation "*Solanum* species plant line" at various places – for example, line 1 of claims 35-39, 41, and 44 as well as the second to last line of claim 41. This limitation does not occur in the specification, abstract or claims-as-filed in the parent application. Thus, the claims as amended comprise NEW MATTER. In response to this rejection, Applicant is required to point to support for the claims or to cancel the new matter.

In the interest of compact prosecution, this limitation is interpreted as meaning any line of a *Solanum* species.

7. Claims 35-48 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention.

The Claims

Claim 1 is drawn to a diploid, fertile, self-compatible *Solanum* species plant line which produces at least 200 grams of tuber per plant and where at least 50% of loci in said plant are homozygous.

Dependent claim 36 requires that the self-compatibility is controlled by the *Sli* gene. Dependent claim 37 requires that the plant comprises an additional agronomically desirable trait. Dependent claim 38 requires a certain amount of fresh weight of foliage. Dependent claim 39 reads on a plant of the line; dependent claim 39 reads on a seed or tuber of the plant.

Claim 41 is drawn to a method of producing a *Solanum* line that is diploid, fertile, self-compatible, produces at least 200 grams fresh weight of tubers per plant, and at least 50% of the genomic loci in said plant line are homozygous in six steps:
(1st) providing a first potato plant that is diploid, fertile, self-compatible and essentially homozygous;
(2nd) providing a second potato plan that is a diploid *Solanum* plant with any level of homozygosity;
(3rd) cross-pollinating the two plants to provide a seed and then collecting said seed;
(4th) growing a multiplicity of said seed and selecting a self-compatible plant;
(5th) inbreeding said self-compatible potato plant to said line of said first potato plant 1 to 8 times; and
(6th) selecting within each generation of inbreeding for a fertile offspring plant to produce a diploid, fertile, self-compatible *Solanum* line, that is at least 50% homozygous. The claim does not require that the plant produced has a yield of 200 grams of tuber per plant. Dependent claim 42 requires that the self-compatibility is controlled by the *Sli* gene. Dependent claim 43 requires that the plant used in the second part *supra* has an agronomically desirable trait. Dependent claim 44 reads on a plant line produced by the method.

Claim 45 is similar to claim 41 but requires a certain level of contrasting homozygous loci. Dependent claim 46 requires that the self-compatibility is controlled by the *Sli* gene. Dependent claim 47 requires that the plant used in the second part *supra* has an agronomically desirable trait. Dependent claim 48 reads on a seed produced by the method.

Applicant's Description

Applicant describes deposited lines by breeders reference: AGVD1 (NCIMB 41663), AGVD2 (NCIMB 41664), AGVD3 (NCIMB 41665) and AGVD17 (NCIMB 41765). Spec., pp. 33-35.

In the specification, Applicant describes a breeding process according to the instant invention starting with a self-compatible line, e.g. AGVD1-3 or AGVD17. *Id.*, p. 37. Applicant states that "[n]o or at least little, attention is paid to the agronomical appearance of the plants at this stage." *Id.* One aspect of the recited breeding steps includes the possibility of adding a desired trait, in the instant case, vigor. *Id.*, p. 38. Continuing this aspect, after adding the trait, the hybrid is backcrossed to the self-compatible line while selecting for vigor. *Id.* After adding numerous traits, and performing test crosses, inbred elite diploid breeding lines can be obtained which can be used to produce commercially valuable hybrids. *Id.*, pp. 41-43.

Applicant reviews the steps: develop a "a vigorous fertile homozygous self-compatible diploid potato line;" "a diploid breeding line;" and then an "elite diploid potato breeding line." *Id.*, p. 40. On page 43, Applicant concludes that "[t]he above method now provides a breeding process for the production of homozygous breeding lines or elite breeding lines based on the background of population AGVD1, AGVD2, AGVD3 or

AGVDI7, to which background successively additional alleles are added, thereby providing elite breeding lines wherein desirable traits are stacked." *Id.*, p. 43.

In particular, in Example 1, Applicant describes obtaining the IVP07-1001/4 line from publically available germplasm where the IVP07-1001/4 plants contained the *Sli* gene after repeated selfing. Spec., p. 44. Applicant cites to the 2006 Euphytica publication by Phumichai et al. as well as the 2005 Genome publication by Phumichai et al. regarding the source of the *Sli* gene. *Id.*, p. 44 & p. 57 (*citing* to Phumichai et al. (2005) Genome 48:977-94 and Phumichai et al. (2006) Euphytica 148:227-34 (2006)). Applicant describes that these plants are of no commercial value. *Id.* Applicant crossed these plants to three different diploid donor lines, D1-3, in order to acquire commercially valuable traits carried by the donor lines. *Id.*, pp. 44-45. The F1 cross with D1 was selfed and the progeny deposited as AGVD1, which was estimated as 50% homozygous. *Id.*, p. 45. The F1(D1) was crossed with F1(D2) and the progeny deposited under AGVD2, and this was estimated to be 25% homozygous with respect to alleles originating from IVP07-1001/4. *Id.* The F1(D1) was backcrossed to obtain progeny 25% homozygous with respect to alleles originating from IVP07-1001/4; these were deposited as AGVD3. *Id.*

In Example 2, Applicant describes crossing the *Sli* gene donor with various publically available potato clones designated: IVP97-079-9, IVPAA-096-I8 and SH83-92-488 to generate clones D1, D2 and D3. *Id.*, pp. 47-50. Applicant describes this created a diploid trait donor lines containing the *Sli* gene with enhanced agricultural quality. *Id.* Applicant provides a more detailed description of the potato lines used in Table 6. *Id.*, pp. 57-58.

Applicant describes creating a molecular marker platform, i.e. SNPs, in Example 3. *Id.*, pp. 50-58.

In Example 4, Applicant describes the development of homozygous diploid potato plants and then F1 hybrid progeny, as well as subsequent generations. *Id.*, pp. 58-62. Applicant describes that it is "often stated" that potatoes are very prone to inbreeding depression. *Id.*, p. 60. A cause of this might be deleterious alleles; Applicant potentially identified a few such deleterious alleles, with one allele almost being lethal. *Id.* However, no absolute lethal allele was present. *Id.* (heading at line 10).

Applicant concludes, on page 62, that

> It was found that after two generations of selfings homozygous diploid potato plants were obtained comprising alleles from both parents. Not a single marker out of 36 markers tested provided proof for a lethal allele. Furthermore, plants with more than 84% homozygous loci can be successfully crossed to obtain hybrid plants with at least five heterozygous chromosome regions. This means that hybrid seeds that are essentially uniform and that represent true potato seed have now been produced.

Id., p. 62.

The Scope of the Claims

In contrast to the limited description – four deposited lines – the claimed genus is quite large. Claim 35, for example, reads on any diploid fertile and self-compatible line created from any *Solanum* species that, for example, produces plants with a tuber yield of 200 grams per plant but Applicant only describes by deposit four lines. The method claims also read on any method of creating such a line, but Applicant only describes methods using the *Sli* gene.

To claim a genus under the written description requirement, Applicant is required to describe a representative number of species to reflect the variation within the genus or structures sufficient to define the genus. The factors to be considered include disclosure of complete or partial structure, physical and/or chemical properties, functional characteristics, structure/function correlation, methods of making the claimed product, or any combination thereof.

Applicant Fails to Describe a Representative Number of Species or Structural Features

Applicant clearly provides written description – by deposit – for the deposited lines. But does Applicant describe a representative number of species in comparison to the size of the genus claimed? In the instant application, Applicant only describes by deposit only four potato lines as compositions and for use in the claimed methods. All were created from the same *Sli* donor, the IVP07-1001/4 line, crossed to three diploid donor lines. Spec., p. 44 & pp. 57-58. Applicant cites to Phumichai et al. (2006) as to the source of the *Sli* gene line. *Id.*, p. 44. The IVP07-1001/4 line does not appear to be a *S. tuberosum* line based on a review of the specification and both Phumichai et al. (2005) and Phumichai et al. (2006), but its identity is not directly stated. Applicant does not further characterize or describe the *Sli* gene.

Similarly, Applicant fails to describe the breeding history of the deposited lines. The most detailed descriptions of D1, D2 and D3 are found in Table 6, but Table 6 fails to provide the genetic background of the lines. Spec., p. 57.

Applicant fails to associate any genetic structure required for the practice of the invention.

The Art

The art describes basic the approach of Applicant's invention. For example, in a 2009 publication, Jansky describes first obtaining homozygous diploids using a self-incompatibility inhibitor. Jansky (2009) "Breeding, Genetics, and Cultivar Development," Advances in Potato Chemistry and Technology, ed. J. Singh & L. Kaur, pp. 27-62, 46. Jansky teaches that "[h]ighly homozygous inbreds could be also be used for heterosis breeding. Jansky, p. 46. Jansky teaches that such an approach would be difficult. *Id.*

Phumichai et al. confirms the approach of Jansky, and documents the use of the *Sli* gene in the approach. Phumichai et al. (2005), abstract.

> Potato . . . is an auto- or segmental allo-tetraploid showing tetrasomic inheritance, which results in complicated genetic segregation. Since potatoes are propagated vegetatively, highly heterotic genotypes can be maintained, which facilitates the use of heterosis, an essential factor in potato breeding, particularly for tuber yield. Alternatively, open-pollinated true potato seed (TPS) can be used as a low-cost planting material for potato production.
>
> Highly homozygous inbreds, once amply produced, would be highly useful for the development of TPS varieties with genetic uniformity, as well as for the investigation of heterosis.
>
> A scheme for hybrid breeding using homozygous diploids and subsequent somatic hybridization has been proposed, for which the availability of highly homozygous diploid (or pure) lines is a key for success. In considering the use of pure lines for the development of genetically uniform [true potato seed] varieties, self-fertile, seed-propagated lines are highly desirable, and simple techniques to develop such lines are necessary.

Id., pp. 977-78 (citations omitted).

Finally, in 2016 Jansky et al. published an "Opinion & Policy" article calling for "Reinventing Potato as a Diploid Inbred Line-Based Crop." Jansky et al. (2016) Crop

Sci. 56:1412-22. The authors call for the creation of "diploid inbred lines" (*id.*, p. 1413) with improved, e.g. tuber yield (*id.*, p. 1414) for use in creating inbred pools for use in heterosis. *Id.* The authors devote almost a page to the challenges faced. *Id.*, p. 1415. Then the authors addressed questions that must still be answered. *Id.* pp. 1415-18. One of the approaches cited by Jansky et al. is Dr. Lindhout's work – i.e. the instant claimed invention – which is described as being in the process of ongoing testing. *Id.*, pp. 1417-18. Therefore, approximately seven years after the filing date of the instant application, the problem of creating vigorous inbred diploid *S. tuberosum* plants apparently still exits.

Admissions in the Parent Applicant and the Art Relevant to the Scope of the Pending Claims

The claims read on broad genera of potato lines, but would all potato lines be active in the instant invention? What distinguishes a potato line that has 50% homozygosity and a 200 gram tuber yield from another potato line falling outside the scope of the claims?. In the specification, Applicant states that

> It is often stated that repeated selfings in diploid potato will only result in weak plants due to inbreeding depression. This should be due to numerous deleterious alleles that reduce plant fitness, when these alleles are homozygously present. Therefore, we tested the segregation of 36 markers in the six F3 populations mentioned (see Figure 5).

Spec., p. 60. As discussed above, Applicant summarizes the result of the study as identifying some potentially, deleterious alleles, one of which is "very" deleterious, almost lethal. *Id.*

The declaration by Mr. Lindhout dated 15 August 2016 in the file history of US application serial no. 13/504,156 and supporting letters document how unique the

potato lines used for the instant invention are. For example, one of the letters offered in support of Dr. Lindhout's declaration by Dr. J.D.G. Jones states that

> "[I]n the mid 2000s we attempted to produce hybrid potato ourselves. . . . However, we never succeeded in developing any promising material in the form of fertile homozygotes that could be useful for hybrid breeding and that could tolerate recurrent selfing. . . . We considered that this was due to our inability to cross the Sli- self-compatibility locus into good enough or diverse enough diploid *Solanum* germplasm.

Letter from Dr. Jonathan D.G. Jones (2012), p. 1 (appended to Decl. of Dr. Lindhout 15 August 2016, ("Decl. (2016)")) (underlining added).

In this declaration, Dr. Lindhout summarizes the background art prior to the claimed invention. Decl. (2016), pp. 3-4. In particular, Dr. Lindhout states that

> That as soon as a genotype becomes homozygous for negative alleles of which the negative is not compensated by functional paralogues, such genotypes might show very poor performance and low viability;
> That inbreeding depression is manifested as a reduced fitness with a loss in both yield and fertility;
> That inbreeding depression is mainly due to the presence of many (semi) lethal alleles, and alleles with a deleterious effect, that become visible more frequently at the diploid level when the maximum number of possible alleles is reduced from four to two, resulting in homozygosity of such alleles.
> That the chance of obtaining a progeny plant, by crossing different genotypes, which is not suffering from inbreeding depression in potato is infinitesimally small, and that starting from a heterozygous diploid potato genotypes, the chance of identifying a plant without homozygous deleterious alleles is, in practice, impossible . . .

Decl. (2016), p. 4.

In a similar vein, in a paper filed with the EPO, Applicant also asserted that "every expert in the field can confirm that due to inbreeding depression, the chances of successfully attaining the plant of the invention are close to zero. Hence, the production of such plants is generally considered impossible." EPO Publication No. EP 2 493 286 A2, Remarks to EPO (21 Dec. 2012), p. 8.

Furthermore, on 15 December 2011, approximately two years after the filing date of the instant application, Lindhout et al. published a paper in the Potato Research journal which appears to correspond to the experiments set forth in the instant examples (all five inventors are listed as authors on the paper). Lindhout et al. (2011) Potato Res 54:301-12. The discussion in the paper is instructive. For example, Lindhout et al. describes that

> As a consequence of the high level of unfavourable alleles, techniques aimed at developing homozygotes in one step, although successful as a technology in various crops, certainly do not generate vigorous homozygous potatoes. For instance, if 20 of the 39,000 protein-encoding genes (PGSC 2011) on the potato genome harbor alleles causing severe fitness reduction, the chance to generate vigorous diploid selfed progenies without homozygous unfavourable loci from a parent genotype, that is heterozygous for these 20 loci, is only 0.3%.

Id., p. 303. Lindhout et al. concludes that "inbred lines were developed that combined self-compatibility with good agronomic performance. Although the number of such plants is still low, the progress made in this breeding programme is significant." *Id.*, p. 304. As a possible reason for success, the authors offer that "lethal alleles do not occur at high frequency in our diploid potato germplasm. *Id.*, pp. 308-09. The authors continue that

> there are many loci with minor effects involved in these traits (Quantitative Trait Locus, QTL). Our current research focuses on identifying the crucial QTLs for these traits. The results will be applied in breeding and allow to increase efficiencies by using diagnostic markers for these traits.

Id., p. 309.

Claims 41-48 are included in this rejection largely because the only embodiment taught by Applicant uses the *Sli* gene and the *Sli* gene is not publically available. Although the *Sli* gene is present in Applicant's deposits, the claims do not reference the

deposits. Furthermore, even though the *Sli* gene is present in the deposits, it is not further described.

Conclusion

Applicant's deposited lines are described and novel. However, in view of the above, especially including Applicant's assertion that "the production of such plants is generally considered impossible" *supra*, there is nothing in the claims to distinguish Applicant's deposited lines from other *Solanum* lines which fall outside the scope of the claimed tuber yield except for the tuber yield. Yet the claims read on a broad genus lines, provided they have a yield of 200 g per plant and are diploid and self-compatible. One of ordinary skill in the art is given no criteria in the claims to distinguish an "impossible" plant from and "possible" plant such as found in Applicant's deposited lines. Applicant, for example, fails to describe any distinguishing structure that is necessary and/or sufficient for the instant invention.

Therefore Applicant's invention appears to require both the *Sli* gene as represented by the deposits and other specific *S. tuberosum* diploid plants that are not described except potentially by reference to the deposit. Applicant fails to describe working examples of the broad genus of any other *Solanum* lines, which, if combined with the *Sli* gene, that fall within the scope of the claims. Applicant only describes one *Sli* gene donor line together with only a few diploid donor lines by deposits. Thus, Applicant fails to describe a representative number of species in comparison to the size of the genus claimed. The claims also fail to require any structure / function relationship that may be necessary and/or sufficient for a potato line to successfully be included in the claimed genus.

The specification therefore fails to provide an adequate written description to support the breadth of the claims. Applicant has not demonstrated possession to one of skill in the art of the instant invention as broadly as claimed at the time of filing. Thus one of skill in the art would not believe Applicant to be in possession of the genus as broadly as claimed especially in view of the discussion of the art by, e.g., Applicant before the EPO and the letter of Dr. Jones.

Dependent claims are included in this rejection because none provide further limitations obviating this rejection.

Claim Rejections - 35 USC § 112 (enablement)

8. Claims 35-48 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The invention appears to employ novel plants which comprise the *Sli* gene which gene does not appear to be otherwise publically available. Since the plant is essential to the claimed invention it must be obtainable by a repeatable method set forth in the specification or otherwise be readily available to the public. If the plant is not so obtainable or available, the requirements of 35 USC § 112 may be satisfied by a deposit of the seeds. A deposit of 625 seeds in the form of 25 packets of 25 seeds of each of the claimed embodiments is considered sufficient to ensure public availability. 1460 OG 263 (2019).

The specification does not disclose a repeatable process to obtain the plant and thus it is not apparent if the plant is readily available to the public.

(a) If a deposit is made under the terms of the Budapest Treaty, then a statement, affidavit or declaration by Applicant, or a statement by an attorney of record over his or her signature and registration number, or someone empowered to make such a statement, stating that the instant invention will be irrevocably and without restriction released to the public upon the issuance of a patent, would satisfy the deposit requirement made herein.

(b) If a deposit has not been made under the Budapest Treaty, then in order to certify that the deposit meets the criteria set forth in 37 CFR 1.801-1.809 and MPEP 2402-2411.05, Applicant may provide assurance of compliance by statement, affidavit or declaration, or by someone empowered to make the same, or by a statement by an attorney of record over his or her signature and registration number showing that:

(i) during the pendency of this application, access to the invention will be afforded to the Commissioner upon request;

(ii) all restrictions upon availability to the public will be irrevocably removed upon granting of the patent in accordance with 37 CFR § 1.808(a)(2);

(iii) the deposit will be maintained in a public depository for a period of 30 years or 5 years after the last request or for the effective life of the patent, whichever is longer;

(iv) a test of the viability of the biological material at the time of deposit (see 37 CFR § 1.807); and,

(v) the deposit will be replaced if it should ever become inviable.

In addition, the identifying information set forth in 37 CPR 1.809(d) should be added to the specification, if not already present. See 37 CPR 1.801 - 1.809 [MPEP 2401-2411.05] for additional explanation of these requirements.

Applicant has deposited the seeds (see e.g. pp. 33-34 of the instant specification). Examiner notes that, *inter alia*, a statement, affidavit or declaration by Applicant, or a statement by an attorney of record over his or her signature and registration number, or someone empowered to make such a statement, stating that the instant invention will be irrevocably and without restriction released to the public upon the issuance of a patent is required.

Claim Rejections - 35 USC § 112 (scope of enablement)

9. Claims 35-48 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, because the specification, while being enabling for the deposited cell lines, does not reasonably provide enablement for forms of self-compatibility as broadly as claimed. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The invention features plants with novel characteristics. Since these plant characteristics are essential to the claimed invention they must be obtainable by a repeatable method set forth in the specification or otherwise be readily available to the public. If such a plant is not so obtainable or available, the requirements of 35 USC § 112 may be satisfied by a deposit of the seeds. Deposits under NCIMB accession numbers 41663, 41664, 41665 and 41765 were made. However the claims are not drawn solely to the deposits.

The Claims

Claim 1 is drawn to a diploid, fertile, self-compatible *Solanum* species plant line which produces at least 200 grams of tuber per plant and where at least 50% of loci in said plant are homozygous.

Dependent claim 36 requires that the self-compatibility is controlled by the *Sli* gene. Dependent claim 37 requires that the plant comprises an additional agronomically desirable trait. Dependent claim 38 requires a certain amount of fresh weight of foliage. Dependent claim 39 reads on a plant of the line; dependent claim 39 reads on a seed or tuber of the plant.

Claim 41 is drawn to a method of producing a *Solanum* line that is diploid, fertile, self-compatible, produces at least 200 grams fresh weight of tubers per plant, and at least 50% of the genomic loci in said plant line are homozygous in six steps:
(1st) providing a first potato plant that is diploid, fertile, self-compatible and essentially homozygous;
(2nd) providing a second potato plan that is a diploid *Solanum* plant with any level of homozygosity;
(3rd) cross-pollinating the two plants to provide a seed and then collecting said seed;
(4th) growing a multiplicity of said seed and selecting a self-compatible plant;
(5th) inbreeding said self-compatible potato plant to said line of said first potato plant 1 to 8 times; and
(6th) selecting within each generation of inbreeding for a fertile offspring plant to produce a diploid, fertile, self-compatible *Solanum* line, that is at least 50% homozygous. The claim does not require that the plant produced has a yield of 200

grams of tuber per plant. Dependent claim 42 requires that the self-compatibility is controlled by the *Sli* gene. Dependent claim 43 requires that the plant used in the second part *supra* has an agronomically desirable trait. Dependent claim 44 reads on a plant line produced by the method.

Claim 45 is similar to claim 41 but requires a certain level of contrasting homozygous loci. Dependent claim 46 requires that the self-compatibility is controlled by the *Sli* gene. Dependent claim 47 requires that the plant used in the second part *supra* has an agronomically desirable trait. Dependent claim 48 reads on a seed produced by the method.

The Scope of the Claims

In contrast to the limited description – four deposited lines – the claimed genus is quite large. Claim 35, for example, reads on any diploid fertile and self-compatible line created from any *Solanum* species that, for example, produces plants with a tuber yield of 200 grams per plant but Applicant only has deposited four lines. The method claims also read on any method of creating such a line, but Applicant only describes methods using the *Sli* gene.

The Federal Circuit in *In re Wands* lists eight considerations for determining whether or not undue experimentation would be necessary to practice an invention. *In re Wands*, 858 F2d 731, 738, 8 USPQ2d 1400, 1406 (Fed. Cir. 1988). These factors are: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples of the invention, the nature of

the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art, and the breadth of the claims. *Id.*

Applicant's Teachings

Applicant deposited lines designated by breeders reference and deposit number: AGVD1 (NCIMB 41663), AGVD2 (NCIMB 41664), AGVD3 (NCIMB 41665) and AGVD17 (NCIMB 41765). Spec., pp. 33-35.

In the specification, Applicant teaches a breeding process according to the instant invention starting with a self-compatible line, e.g. AGVD 1-3 or AGVD17. *Id.*, p. 37. Applicant teaches that "[n]o or at least little, attention is paid to the agronomical appearance of the plants at this stage." *Id.* One aspect of the breeding steps taught by the specification includes the possibility of adding a desired trait, such as vigor. *Id.*, p. 38. After adding such a trait, the hybrid is backcrossed to the self-compatible line while selecting for, e.g. vigor. *Id.* After adding numerous traits, and performing test crosses, inbred elite diploid breeding lines can be obtained which can be used to produce commercially valuable hybrids. *Id.*

Applicant reviews the steps: develop a "a vigorous fertile homozygous self-compatible diploid potato line;" "a diploid breeding line;" and then an "elite diploid potato breeding line." *Id.*, p. 40. On page 43, Applicant concludes that "[t]he above method now provides a breeding process for the production of homozygous breeding lines or elite breeding lines based on the background of population AGVD1, AGVD2, AGVD3 or AGVDI7, to which background successively additional alleles are added, thereby providing elite breeding lines wherein desirable traits are stacked." *Id.*, p. 43.

In describing the experiment that underlie the claimed invention, Applicant teaches obtaining the IVP07-1001/4 line from publically available germplasm where the IVP07-1001/4 plants contained the *Sli* gene after repeated selfing in Example 1. Spec., p. 44. Applicant cites to the 2006 Euphytica publication by Phumichai et al. (Euphytica 148:227-34 (2006)) as well as the 2005 Genome publication by Phumichai et al. Genome 48:977-94 (2005)) regarding the source of the *Sli* gene. *Id.*, p. 44 & p. 57 Applicant teaches that these plants are of no commercial value. *Id.* Applicant crossed these plants to three different diploid donor lines, D1-3, in order to acquire commercially valuable traits carried by the donor lines. *Id.*, pp. 44-45. The F1 cross with D1 was selfed and the progeny deposited as AGVD1, which was estimated as 50% homozygous. *Id.*, p. 45. The F1(D1) was crossed with F1(D2) and the progeny deposited under AGVD2, and this was estimated to be 25% homozygous with respect to alleles originating from IVP07-1001/4. *Id.* The F1(D1) was backcrossed to obtain progeny 25% homozygous with respect to alleles originating from IVP07-1001/4; these were deposited as AGVD3. *Id.*

In Example 2, Applicant teaches crossing the *Sli* gene donor with various publically available potato clones designated: IVP97-079-9, IVPAA-096-I8 and SH83-92-488 to generate clones D1, D2 and D3. *Id.*, p. 47. Applicant teaches this created a diploid trait donor lines containing the *Sli* gene with enhanced agricultural quality. *Id.*, pp. 46-50. Applicant provides a more detailed description of the potato lines used in Table 6. *Id.*, pp. 57-58.

Applicant teaches creating a molecular marker platform, i.e. SNPs, in Example 3. *Id.*, pp. 50-58.

In Example 4, Applicant teaches the development of homozygous diploid potato plants and then F1 hybrid progeny, as well as subsequent generations. *Id.*, pp. 58-62. Applicant teaches that it is "often stated" that potatoes are very prone to inbreeding depression. *Id.*, p. 60. A cause of this might be deleterious alleles; Applicant potentially identified a few such deleterious alleles, with one allele almost being lethal. *Id.*

In Example 4, Applicant teaches the development of homozygous diploid potato plants and then F1 hybrid progeny, as well as subsequent generations. *Id.*, pp. 58-62. Applicant describes that it is "often stated" that potatoes are very prone to inbreeding depression. *Id.*, p. 60. A cause of this might be deleterious alleles; Applicant potentially identified a few such deleterious alleles, with one allele almost being lethal. *Id.* However, no absolute lethal allele was present. *Id.* (heading at line 10).

Applicant concludes, on page 62, that

> It was found that after two generations of selfings homozygous diploid potato plants were obtained comprising alleles from both parents. Not a single marker out of 36 markers tested provided proof for a lethal allele. Furthermore, plants with more than 84% homozygous loci can be successfully crossed to obtain hybrid plants with at least five heterozygous chromosome regions. This means that hybrid seeds that are essentially uniform and that represent true potato seed have now been produced.

Id., p. 62.

The Art

The art generally teaches this breeding strategy, or similar strategies. For example, Jansky teaches first obtaining homozygous diploids using a self-incompatibility inhibitor. J Jansky, "Breeding, Genetics, and Cultivar Development," Advances in Potato Chemistry and Technology, ed. J. Singh & L. Kaur, pp. 27-62 (June, 2009), p. 46. Jansky teaches that "[h]ighly homozygous inbreds could be also be used

for heterosis breeding. Jansky, p. 46. Jansky teaches that such an approach would be difficult. *Id.*

Phumichai et al. confirms the approach of Jansky, and documents the use of the *Sli* gene in the approach. Phumichai et al., Genome 48:977-84 (2005), abstract.

> Potato . . . is an auto- or segmental allo-tetraploid showing tetrasomic inheritance, which results in complicated genetic segregation. Since potatoes are propagated vegetatively, highly heterotic genotypes can be maintained, which facilitates the use of heterosis, an essential factor in potato breeding, particularly for tuber yield. Alternatively, open-pollinated true potato seed (TPS) can be used as a low-cost planting material for potato production.
>
> Highly homozygous inbreds, once amply produced, would be highly useful for the development of TPS varieties with genetic uniformity, as well as for the investigation of heterosis.
>
> A scheme for hybrid breeding using homozygous diploids and subsequent somatic hybridization has been proposed, for which the availability of highly homozygous diploid (or pure) lines is a key for success. In considering the use of pure lines for the development of genetically uniform [true potato seed] varieties, self-fertile, seed-propagated lines are highly desirable, and simple techniques to develop such lines are necessary.

Id., pp. 977-78 (citations omitted).

Finally, in 2016 Jansky et al. published an "Opinion & Policy" article calling for "Reinventing Potato as a Diploid Inbred Line-Based Crop." Jansky et al. (2016) Crop Sci. 56:1412-22. The authors call for the creation of "diploid inbred lines" (*id.*, p. 1413) with improved, e.g. tuber yield (*id.*, p. 1414) for use in creating inbred pools for use in heterosis. *Id.* The authors devote almost a page to the challenges faced. *Id.*, p. 1415. Then the authors addressed questions that must still be answered. *Id.* pp. 1415-18. One of the approaches cited by Jansky et al. is Dr. Lindhout's work – i.e. the instant claimed invention – as undergoing ongoing testing. *Id.*, pp. 1417-18. Therefore,

approximately seven years after the filing date of the instant application, the problem of creating vigorous inbred diploid *S. tuberosum* plants apparently still exits.

Claims 41-48 are included in this rejection largely because the only embodiment taught by Applicant uses the *Sli* gene and the *Sli* gene is not publically available. Although the *Sli* gene is present in Applicant's deposits, the deposits have not been perfected with respect to this application.

Revisiting the Scope of the Claims

Applicant claims a large genus of *Solanum* plant lines. Both Jansky and Spooner et al. describes the breadth of the of the claimed genus as extremely large. *See*, e.g., Jansky (2009) "Breeding, Genetics, and Cultivar Development," pp. 27-29; Spooner et al. (2014) Bot Rev 80:283-383, 286-94, Table 1. Applicant's invention in claim 35, for example, reads on any potential potato line that is diploid, fertile, self-compatible and where the potato line has a yield of 200 g per plant.

In view of the arguments presented above, success in practicing Applicant's claimed invention is unpredictable and depends on the genotype of the potato lines. Therefore, extensive guidance would be required to enable the invention as broadly as claimed. Such guidance is not provided and the claims encompass a broad, unpredictable genus.

The specification therefore does not enable one skilled in the art to make and use members of the species of the claimed genus other than the deposited lines. *See*, for example, MPEP 2164.06(a)(II), citing to *Ex parte Jackson*, 217 USPQ 804, 806 (Bd. App. 1982). The issue before the Board in *Ex parte Jackson* was:

> The issue squarely raised by this rejection is whether or not a description of several newly discovered strains of bacteria having one particularly desirable metabolic property in terms of the conventionally measured culture characteristics and a number of metabolic and physiological properties would enable one of ordinary skill in the relevant art to independently discover additional strains having the same specific, desirable metabolic property, i.e., the production of a particular antibiotic.
>
> To state the issue somewhat differently, it is whether a verbal description of a new species would enable one of ordinary skill in the relevant art to obtain strains of that species over and above the specific strains made available through deposit in one of the recognized culture depositories.

Ex parte Jackson, 217 USPQ 804, 806 (Bd. App. 1982). In the instant application, the issue is similar to the one before the Board in that Applicant made deposits with unique characteristics but not taught how to make and use other lines similar to the deposited lines. In *Ex parte Jackson*, the Board held that the deposit, without a detailed explanation of how to obtain more species, did not enable the generic claims. *Id.*, p. 808. The board found that "the degree of experimentation involved in locating new [organisms] apart from deposited cultures is undue in light of the enablement requirement of 35 U.S.C. 112." *Id.* The *Jackson* decision was considered and distinguished by the Federal Circuit in the *Wands* decision in view of the routine experimentation required to obtain hybridomas that were before the bar in the Wands application. *In re Wands*, 858 F2d 735-37, 8 USPQ2d at 1403-04. Therefore, in particular in view of the *Ex parte Jackson* decision, the generic scope of the claims reading on the genus as set forth above is not enabled. Obtaining further species falling within the scope of the generic claims would require undue experimentation.

Furthermore, in 1966, the Supreme Court held that "[t]he basic quid pro quo contemplated by the Constitution and the Congress for granting a patent monopoly is

the benefit derived by the public from an invention with substantial utility. Unless and until a process is refined and developed to this point-where specific benefit exists in currently available form, there is insufficient justification for permitting an application to engross what may prove to be a broad field. *Brenner v. Manson*, 383 U.S. 519, 534-35, 148 USPQ 689, 695 (1966).

Dependent claims are included in this rejection because none provide further limitations obviating this rejection.

Additionally, Applicant provides minimal teachings and/or guidance regarding the *Sli* gene, which appears to be an essential part of the claimed invention.

Claim Rejections - 35 USC § 112(d)

The following is a quotation of 35 U.S.C. 112(d):

> (d) REFERENCE IN DEPENDENT FORMS.—Subject to subsection (e), a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

The following is a quotation of pre-AIA 35 U.S.C. 112, fourth paragraph:

> Subject to the following paragraph [i.e., the fifth paragraph of pre-AIA 35 U.S.C. 112], a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

9. Claim 37 is rejected under 35 U.S.C. 112(d) or pre-AIA 35 U.S.C. 112, 4th paragraph, as being of improper dependent form for failing to further limit the subject matter of the claim upon which it depends, or for failing to include all the limitations of the claim upon which it depends. Claim 37 depends from claim 35 but requires an additional agronomically desirable trait. Art that could apply to the plant of claim 37 but might not apply to the plant of claim 35. Thus claim 37 is broader in scope than claim 35 and fails to further limit the claim.

Applicant may cancel the claim(s), amend the claim(s) to place the claim(s) in proper dependent form, rewrite the claim(s) in independent form, or present a sufficient showing that the dependent claim(s) complies with the statutory requirements.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

10. Claims 41-48 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Phumichai et al. (2005) Genome 48:977-94 in view of Maris (1990) Euphytica 46:15-33 in further view of Watanabe et al. (1996) Breed Sci 46:329-36.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

a. Determining the scope and contents of the prior art.

b. Ascertaining the differences between the prior art and the claims at issue.

c. Resolving the level of ordinary skill in the pertinent art.

d. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claim 41 is drawn, according to the preamble, to a method of producing a *Solanum* line that is diploid, fertile, self-compatible, produces at least 200 grams fresh weight of tubers per plant, and at least 50% of the genomic loci in said plant line are homozygous. Claim 41 has in six steps:

(1st) providing a first potato plant that is diploid, fertile, self-compatible and essentially homozygous;

(2nd) providing a second potato plan that is a diploid *Solanum* plant with any level of homozygosity;

(3rd) cross-pollinating the two plants to provide a seed and then collecting said seed;

(4th) growing a multiplicity of said seed and selecting a self-compatible plant;

(5th) inbreeding said self-compatible potato plant to said line of said first potato plant 1 to 8 times; and

(6th) selecting within each generation of inbreeding for a fertile offspring plant to produce a diploid, fertile, self-compatible *Solanum* line, that is at least 50% homozygous.

Maris teaches breeding potatoes from diploid parental lines, e.g. phureja PI 195198, and thus teaches a diploid, fertile, self-compatible and essentially homozygous potato line. Maris, pp. 15 & 17. Applicant defines "essentially homozygous" as requiring at least 25% homozygosity and thus it is reasonable to interpret the lines used by Maris as being "essentially homozygous" according to Applicant's standard. None of the steps of the method actually require that any of the plants have a tuber yield of 200 grams, but in any case Maris teaches plants that have an average tuber yield of at least 200 g/plant. See, e.g., Maris, Table 8, pp. 26-28.

Phumichai et al. (2005) teaches developing diploid potato clones based on (1) the diploid clone 97H32-6 and (2) two advanced diploid clones: WB922236-2 and WP922236-3. Phumichai et al. (2005), p. 978. Phumichai et al. teaches crossing them.

Id., p. 979. Phumichai et al. teaches using the *Sli* gene to obtain such self-compatible potato lines.

Other than the starting material, the steps in claim 41 are merely routine backcrossing steps. E.g., Phumichai et al. (2005), entire article. Therefore claims 41-42 and 44 are obvious.

Dependent claims 42 and 46 require that the self-compatibility is controlled by the *Sli* gene. Dependent claims 43 and 47 require crossing in an agronomically desirable trait. Dependent claim 44 and 48 read on a plant line or seed produced by the methods.

Given that Phumichai et al. measure a decrease in heterozygosity of 38% (p. 982) teach berries and seeds (pp. 978-79), claims 45-46 and 48 are obvious.

Watanabe et al. teaches the value of introgressing an agronomically desirable trait, such as nematode resistance, into a diploid breeding stock. See, e.g., Watanabe et al., p. 329. Thus claims 43 and 47 are obvious

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.
Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

The USPTO internet Web site contains terminal disclaimer forms which may be used. Please visit http://www.uspto.gov/forms/. The filing date of the application will determine what form should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

11. Claims 35-48 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 3-6, 16, 18-21, 29-30, 32, 35, 37-39 and 42-43 of copending application no. 13/504,156. Although the conflicting claims are not identical, they are not patentably distinct from each other. This is a provisional double patenting rejection since the conflicting claims have not yet been issued as a US patent although they have been allowed.

The instant application is a continuation where Applicant is seeking broader coverage than the issued claims. As such the instant pending claims represent genera encompassing the species claims in the 13/504,156 application. Thus the pending claim are obvious in view of the allowed claims.

Conclusion

12. No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to RUSSELL T BOGGS whose telephone number is (571)272-2805. The examiner can normally be reached on Monday - Friday, 0800 to 1830 Mtn.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Shubo (Joe) Zhou, can be reached on 571-272-0724. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/RUSSELL T BOGGS/
Examiner, Art Unit 1663

Request for Information under 37 CFR § 1.105

Applicant and the assignee of this application are required under 37 CFR § 1.105 to provide the following information that the examiner has determined is reasonably necessary to the examination of this application.

This request is being made for the following reasons:

Applicant is claiming novel potato lines but the instant specification is silent about what starting materials and methods were used to produce the plants. The requested information is required to make a meaningful and complete search of the prior art.

In response to this requirement, if known, please provide answers to each of the following interrogatories eliciting factual information:

(i) Please supply the breeding methodology and history regarding the development of the instant variety.

a) Such information should include the all the designations / denominations used for the original parental lines.

b) Information should also include information pertaining to the public availability of the original parental lines.

c) The breeding method used should be set forth such as whether single seed descent, bulk method, backcross method, or some other method was used.

d) The filial generation in which the instant plant was chosen should be set forth.

e) Information pertaining to the homozygosity or heterozygosity of the parents as well as the instant plant should be set forth.

f) Are there any patent applications or patents in which sibs of the instant plant are claimed? If so, please set forth their serial numbers as well as the names of the sibs.

If Applicant views any or all of the above requested information as a Trade Secret, then Applicant should follow the guidance of MPEP § 724.02 when submitting the requested information.

In responding to those requirements that require copies of documents, where the document is a bound text or a single article over 50 pages, the requirement may be met by providing copies of those pages that provide the particular subject matter indicated in the requirement, or where such subject matter is not indicated, the subject matter found in applicant's disclosure. Please indicate where the relevant information can be found.

The fee and certification requirements of 37 CFR § 1.97 are waived for those documents submitted in reply to this requirement. This waiver extends only to those documents within the scope of this requirement under 37 CFR § 1.105 that are included in the applicant's first complete communication responding to this requirement. Any supplemental replies subsequent to the first communication responding to this requirement and any information disclosures beyond the scope of this requirement under 37 CFR § 1.105 are subject to the fee and certification requirements of 37 CFR § 1.97.

The applicant is reminded that the reply to this requirement must be made with candor and good faith under 37 CFR § 1.56. Where the applicant does not have or cannot readily obtain an item of required information, a statement that the item is

unknown or cannot be readily obtained may be accepted as a complete reply to the requirement for that item.

This requirement is an attachment of the enclosed Office action. A complete reply to the enclosed Office action must include a complete reply to this requirement. The time period for reply to this requirement coincides with the time period for reply to the enclosed Office action.

Notice of References Cited	Application/Control No. 16/541,071	Applicant(s)/Patent Under Reexamination Lindhout et al.	
	Examiner RUSSELL T BOGGS	Art Unit 1663	Page 1 of 3

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NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
*	U	Phumichai et al. (2006) Euphytica 148:227-34
*	V	Phumichai et al. (2005) Genome 48:977-94
*	W	J Jansky (2009) "Breeding, Genetics, and Cultivar Development," Advances in Potato Chemistry and Technology, ed. J. Singh & L. Kaur, pp. 27-62, 46
*	X	Lindhout et al. (2011) Potato Res 54:301-12

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
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*	U	Jansky et al. (2016) Crop Sci. 56:1412-22
*	V	Decl. of Dr. Lindhout 15 August 2016 in application serial no. 13/504,156
*	W	EPO Publication No. EP 2 493 286 A2, Remarks to EPO (21 Dec. 2012)
*	X	Declaration by Mr. Lindhout dated 15 August 2016 in the file history of US application serial no. 13/504,156

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*	U	Spooner et al. (2014) Bot Rev 80:283-383
*	V	Maris (1990) Euphytica 46:15-33
*	W	Watanabe et al. (1996) Breed Sci 46:329-36
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
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CPC - Searched*		
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NPL search as per attached beginning	11/23/2019	RTB
EAST search as per attached	11/27/2019	RTB
STN search as per attached	11/23/2019	RTB

Interference Search			
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Toward the development of highly **homozygous** diploid **potato** lines using the self-compatibility controlling *Sli* gene

C Phumichai, M Mori, A Kobayashi, O Kamijima... - ..., 2005 - NRC Research Press

... Ainsi, des pommes de terre largement **homozygotes** et reproduites via les graines ont été ... of heterozygosity reduction and identify hetero- zygous loci that resist universal **homozygosity**; and (iii) to discuss the usefulness of Sli gene in developing of highly **homozygous** lines of ...

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Inheritance study of tuber glycoalkaloids in a **wild potato**, *Solanum chacoense* bitter

GD McCollum, SL Sinden - American **Potato** Journal, 1979 - Springer

... Hawkes et Hjerting (5), a diploid, self- incompatible **wild** species of Argentina and adjoining ... **homozygosity** for com- mersonine or chac/- to indicate either heterozygosity or **homozygosity** at a ... allelic (or completely linked in repulsion), all such F2's would be **homozygous** sol~sol ...

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M6: A diploid **potato** inbred line for use in breeding and genetics research

SH Jansky, YS Chung .. - Journal of Plant ..., 2014 - dl.sciencesocieties.org

... Resistance is likely due to an ortholog of the tomato Ve gene, as M6 is **homozygous** for a ... All F 2 populations are being self-pollinated to **homozygosity** to create recombinant inbred line populations, which will ... Significance of **wild** species and primitive forms for **potato** breeding ...

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EL Camadro, RW Masuelli - Sexual Plant Reproduction, 1995 - Springer

... as in acl, is controlled by two inde- pendent loci, carrying in **homozygosity** the alleles "1 ... 750 Camadro EL, Masuelli RW, Cortds MC (1992) Haploids of the **wild** tetraploid **potato** ... uacidn Ing Agr FCA Balcarce, UNMP, Argentina Hawkes JG (1990) The **potato**: evolution, biodiversity ...

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Panglobal distribution of a single clonal lineage of the Irish **potato** famine fungus

SB Goodwin, BA Cohen, WE Fry - Proceedings of the ..., 1994 - National Acad Sciences

... are two potential phenotypes at a locus: +, if the locus is heterozygousor **homozygous** for the ... **Homozygotes** can be distinguished from heterozy- gotes by band intensity (this has been verified for ... 2), or from heterozy- gosity to **homozygosity** at one of the allozyme loci, were the ...

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SH Jansky, GL Yerk, SJ Peloquin - Plant Breeding, 1990 - Wiley Online Library

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Assessing SNPs Versus RAPDs for Predicting Heterogeneity and Screening Efficiency in **Wild Potato** (*Solanum*) Species

J Bamberg, A Del Rio, J Coombs... - American journal of **potato** ..., 2015 - Springer

... of the mutant SNP alleles identified in tuberosum, and thus have mostly **homozygous wild**-type SNP ... Evaluation of **wild** Solanum species for resistance to the US-8 genotype of Phytophthora ... American Journal of **Potato** Research 78: 159–165.CrossRefGoogle Scholar. Felcher, KJ ...

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DM Spooner, M Ghislain, R Simon, SH Jansky... - The botanical ..., 2014 - Springer

... Indigenous cultivated (landrace) **potatoes** and **wild potato** species, all classified as Solanum section Petota, are widely used for **potato** improvement ... Landrace **potatoes** and **wild potato** species, all classified as Solanum section Petota, are widely used for **potato** improvement ...

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potato **haploids** wild **species germplasm**

inheritance study wild potato

solanum acaule bitt wild potato

endosperm balance number wild potato

genetic model wild potato

The effects of certain environmental factors on tuberization in the **wild potato**, *Solanum commersonii*

GE Davis - American Journal of Potato Research, 1941 - Springer

... the efficacy of a low night temperature level in bringing about a better **yield** of **tubers** per **gram** of top weight, although as shown above a larger mean **yield** was produced ... regardless of day length or day temperature, was more limiting to top growth than to **tuber** growth and ...

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Germplasm enhancement with **potato** haploids

SA Hermundstad, SJ Peloquin - Journal of Heredity, 1985 - academic.oup.com

... with small **tubers** 9 0 100 13 18 60 50 33 14 large **tubers** 45 60 0 38 27 20 25 17 21 ... However, parent-family correlations for total **yield** were nonsignificant ... Selec- tion for maturity, **tuber** weight, and **tuber** ap- pearance in the hybrids will be carried out on the first **tuber** generation ...

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Overriding the co-limiting import of carbon and energy into **tuber** amyloplasts increases the starch content and **yield** of transgenic **potato** plants

L Zhang, RE Häusler, C Greiten... - Plant biotechnology ..., 2008 - Wiley Online Library

... **wild**-type. Furthermore, the question was raised as to whether the increase in **tuber** and starch **yield** observed in **tubers** of 10-week-old double transformants occurred continuously during **tuber** development. Data gathered ...

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Mexico, 1988 **potato** germplasm collecting expedition and utility of the Mexican **potato** species

DM Spooner, J Bamberg, JP Hjerling, J Gómez - American Potato Journal, 1991 - Springer

... but the one collection with seeds, and the great quan- tity of **tubers** collected should ... Use of Phureja and haploids to en- hance the **yield** of cultivated tetraploid **potatoes** ... Interactions between **wild** **tuber**-bearing Solanum species and Phytophthora infestans (Mont) de Bary in a ...

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Protocol for DNA extraction from **potato tubers**

EG Wulff, S Torres, EG Vigil - Plant molecular biology reporter, 2002 - Springer

... A method to extract high-quality DNA from **potato tubers** was developed and tested on 3 **wild** **potato** ... The average **yield** of extracted DNA varied from 10–30 µg of DNA per **gram** of processed ... DNA extraction LM-PCR **potato** Solanum **tuber**. Download to read the full article text ...

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Research in*Solanum* spp. of sources of resistance to the **potato tuber** moth*Phthorimaea operculella* (Zeller)

S Arnone, S Musmeci, L Bacchetta, N Cordischi... - Potato Research, 1998 - Springer

... The evaluation of **tuber** infestation could be influcnced by the **yield** differences among ... Alkaloid content of the **tubers** of interspecific hybrids, however, must be thoroughly evaluated ... Breeding for resistance to **potato tuber** moth, Phthorimaea operculella (Zeller), in diploid **potatoes** ...

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Evaluation of **tuber** traits of 10, 2x (2EBN) **wild** species through haploid× **wild** species hybrids

GL Yerk, SJ Peloquin - American Potato Journal, 1989 - Springer

... for countries which grow two crops per year or countries which harvest **tubers** for ex ... Haploid Atl 309 gave the best mean general **tuber** appearance over all the families in ... Evaluation of **yield** and other agronomic characteristics of TPS families and advanced clones from different ...

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Evaluation of **tuber**-bearing*Solanum* species for nitrogen use efficiency and biomass partitioning

M Errebhi, CJ Rosen, FI Lauer, MW Martin... - ... Journal of Potato ..., 1999 - Springer

... to roots plus nontuberized stolons, 39% to shoots, 14% to fruits, and 38% to **tubers** ... breeding programs have been able to capitalize on the diversity of **tuber**-bearing Solanum ... and Glass (1981) who suggested that for genotypic variability studies, the **yield** component should ...

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Sucrose transporter StSUT4 from **potato** affects flowering, tuberization, and shade avoidance response

IA Chincinska, J Liesche, U Krügel, J Michalska... - Plant ..., 2008 - Am Soc Plant Biol

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Advantages of **Wild** Diploid *Solanum* Species Over Cultivated Diploid Relatives in **Potato** Breeding Programs

SH Jansky, SJ Peloquin - Genetic Resources and Crop Evolution, 2006 - Springer

... In contrast, **tuber** dormancy is short in Phureja Group (DeMaine 1978 ... dormancy is desirable in temperate North American and European regions that require **tubers** to be ... Heterosis for **yield** fol- lowing USP has been documented thoroughly using both haploid Tuberosum Group ...

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Solanum tuberosum Group Phureja - Wikipedia

https://en.wikipedia.org › wiki › Solanum_tuberosum_Group_Phureja

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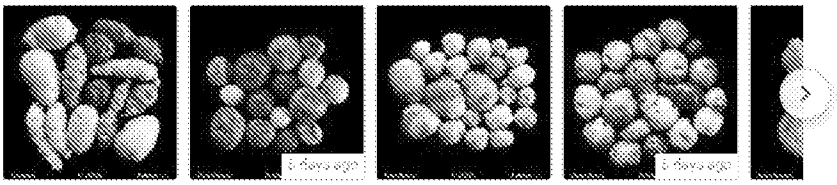
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Cryptic improvement for fertility by continuous selfing of diploid **potatoes** using *Sli* gene

C Phumichai, K Hosaka - Euphytica, 2006 - Springer

... genetic uniformity, and heterosis breeding of the **potato** (Birhman & Hosaka, 2000 ... Although diploid **potatoes** can be continuously selfed using the Sli gene, the actual approach to ho- mozygosity during selfing is ... (2005) monitored the inbreed- ing process from a clone **97H32**-6 to ...

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Toward the development of highly homozygous diploid **potato** lines using the self-compatibility controlling *Sli* gene

C Phumichai, M Mori, A Kobayashi, O Kamijima... - ..., 2005 - NRC Research Press

... diploid **potato** lines using the self-compatibility ... Abstract: Cultivated diploid **potatoes** (2n = 2x = 24) are self-incompatible, but can be altered to become self- compatible using the Sli gene. Previously, a diploid clone **97H32–6** was selfed up to S3 using the Sli gene ...

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DNA methylation in diploid inbred lines of **potatoes** and its possible role in the regulation of heterosis

S Nakamura, K Hosaka - Theoretical and Applied Genetics, 2010 - Springer

... The Sli gene was introduced by crossing into cultivated diploid **potatoes** (Birhman and Hosaka 2000) and ... 1. A diploid clone **97H32**-6 (composed of 75% cultivated and 25% wild germplasm with ... Two series of diploid **potato** inbreds A and B were developed from a diploid **potato** ...

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Expression of *S*-locus inhibitor gene (*Sli*) in various diploid **potatoes**

C Phumichai, Y Ikeguchi-Samitsu, M Fujimatsu... - Euphytica, 2006 - Springer

... Phumichai et al. (2005) reported that the percentage selfers in its selfing generations from **97H32**-6 (the ... Hosaka, K., 1986. Who is the mother of the **potato**? – restriction endonuclease analysis of chloroplast DNA of cultivated **potatoes**. Theor Appl Genet 72: 606–618 ...

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Breakdown of a hybridization barrier between *Solanum pinnatisectum* Dunal and **potato** using the *S* locus inhibitor gene (*Sli*)

R Sanetomo, S Akino, N Suzuki, K Hosaka - Euphytica, 2014 - Springer

... Clones of the 9H27 family, which are S 7 generation plants from **97H32**-6, are ... to use in breeding because of strict reproductive barriers with cultivated **potatoes** (Hawkes 1958 ... To overcome reproductive barriers with **potato**, a variety of techniques have been employed such as ...

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Potato *Virus Y* Resistance Gene, Ry_{chc}, Mapped to the Distal End of **Potato** Chromosome 9

M Sato, K Nishikawa, K Komura, K Hosaka - Euphytica, 2006 - Springer

... a random genomic DNA library of S. phureja 1.22 (Hosaka & Spooner, 1992), which have been localized on this **potato** genome (Hosaka ... female with six male genotypes (Table 2). Only three cross combinations set berries and produced seeds: 40 seeds from **97H32**-6 × 98H20 ...

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Sexual introgression of the late blight resistance gene *Rpi-blb3* from a Mexican wild diploid species *Solanum pinnatisectum* Dunal into **potato** varieties

R Sanetomo, I Habe, K Hosaka - Molecular breeding, 2019 - Springer

... 2x genotype. **97H32**-6 ... Chromosome-doubled 6x F 1 hybrids were backcrossed to cultivated **potatoes** from 2015 to 2017 ... (2011) was used to detect resistance genes to cyst nematode (H1), **Potato** virus X (Rx1), **Potato** virus Y (Ry chc), and late blight (R1 and R2) ...

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Protein having glycoalkaloid biosynthetic enzyme activity and gene encoding the same

N Umemoto - US Patent App. 14/358,568, 2015 - Google Patents

... 16, 2009 (reported by Asahi.com). **Potatoes** are usually a safe food because they are managed such that the content of glycoalkaloid is maintained at 20 mg/100 g or less by storing the tuber of **potato** in a dark place etc. However ...

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Plant having suppressed expression of glycoalkaloid biosynthetic enzyme gene or altered activity of the enzyme

N Umemoto - US Patent 9,765,353, 2017 - Google Patents

... However, in consideration of the risk of such a poisoning accident described above, reducing glycoalkaloids in **potato** is a matter of concern to all of the persons who deal with **potatoes** such as the breeding, production, storage, transportation, sale, and purchase of **potatoes**, but ...

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... やすさから実験材料として利用してきた前項の品種 「サッシー」も同じく細胞質優性不稔である．そのため日本のジャガイモ育種団体で作出された花粉稔性の高い 系統で自殖できる二倍体種である**97H32-6(17)** と品種「な がさき黄金 ... 5) Solynta: Hybrid **potato** breeding, http://solynta.com/, 2018 ...

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/R.T.B/	21	LIPPMAN et al., Trends Genet 23(2):61-66.	☐
/R.T.B/	22	BRADSHAW et al., (2006) Potato Res 49:49-65.	☐
/R.T.B/	23	STOKSTAD, "The New Potato: Breeders seek a breakthrough to help farmers facing an uncertain future," Science, Vol. 363, Issue 6427, pp. 574-577, Feb. 8, 2019.	☐
/R.T.B/	24	JANSKY et al., "M6: A Diploid Potato Inbred Line for Use in Breeding and Genetics Research," J. Plant Registrations (February 21, 2014).	☐
/R.T.B/	25	HANNEMAN, "Self Fertility in Solanum." Am Potato J 62: 428-429 (1985).	☐
/R.T.B/	26	HOSAKA et al., "Genetics of self-compatibility in a self-incompatable wild diploid potato species Soianum chacoense. 2. Localization of an S locus inhibitor (Sii) gene on the potato genome using DNA markers," Euphhytica 103:265-271 (1998).	☐
/R.T.B/	27	WANG et al., (NZ J Crop Hort Sci 28:1-8 (2000)).	☐
/R.T.B/	28	PHUMICHAI et al., Euphytica 148:227-34 (2006).	☐
/R.T.B/	29	JOHNSTONE et al., "Chromosome Doubling in Potatoes Induced by Colchicine Treatment," American Journal of Potato Research, Vol. 16, No. 11, pages 288-304, November 1, 1939.	☐
/R.T.B/	30	RAMULU et al., "Mitotic blocking, micronucleation, and chromosome doubling by oryzalin, amiprophos-methyl, and colchicine in potato." Protoplasma, Vol. 160, No. 2-3, pages 65-71, June 1, 1991.	☐
/R.T.B/	31	SPOONER et al., Bot Rev 80:283-383 (2014) (Year: 2014).	☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	
	Filing Date	
	First Named Inventor	Willem Hendrik Lindhout
	Art Unit	
	Examiner Name	
	Attorney Docket Number	VONL:004USC1

/R.T.B/	32	VEILLEUX, Sol CAP News 3(2):4-4 (2011) (Year: 2011).	☐
/R.T.B/	33	LINDHOUT et al., Potato Res 54:301-12 (2011).	☐
/R.T.B/	34	WATANABE et al., Breed Sci 46:329-36 (1996).	☐
/R.T.B/	35	UIJTEWAAL et al., Euphytica 36: 745-53 (1987).	☐
/R.T.B/	36	EP 2 493 286 A2, Remarks to EPO (21 Dec. 2012).	☐
/R.T.B/	37	BAIRU et al., Plant Growth Regul 63:147-73 (2011).	☐
/R.T.B/	38	Merriam-Webster, "as", accessed 2 December 2016.	☐
/R.T.B/	39	MULLINS et al., Trends Plant Sci 11(5):254-60 (2006).	☐
/R.T.B/	40	JANSKY et al., Crop Sci. 56:1412-22 (2016).	☐
/R.T.B/	41	TROFIMOV et al., Translated Title: "Utilizing Diploid Varieties and Dihaploids to Intensify Potato Selection and How to Better Preserve The Viability of The Pollen of Various Species of Gourds", Translated Journal Title: Potatoes and Vegetables 6 (2007) (English translation).	☐
/R.T.B/	42	TROFIMOV et al., "Ispol'zovaniye Diploidnykh Vidov I Digaploidov Dlya Intensifikatsii Selektsii Kartofelya And Kak Lushche Sokhranit' Zhiznesposobnost' Pylytsy Raznykh Vidov Tykvy", Kartofel' I Ovoshchi 6 (2007) (Russian).	☐

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/R.T.B/	43	MARIS, (Euphytica 46:15-33 (1990).	☐
/R.T.B/	44	SIMMONDS, Potato Res 40:191-214 (1997).	☐
/R.T.B/	45	RAIMONDI et al., Euphytica 132(3):287-95 (2003).	☐
/R.T.B/	46	DINU et al., Intl J Plant Breed Res 110(3):403-15 (2003).	☐
/R.T.B/	47	VISKER et al., Euphytica 137(3):311-23 (2004).	☐
/R.T.B/	48	BANI-AAMEUR et al., Euphytica 68(3):169-79 (1993).	☐
/R.T.B/	49	International Search Report relating to corresponding PCT/NL2010/050716, issued June 8, 2011.	☐
/R.T.B/	50	International Preliminary Report on Patentability relating to corresponding PCT/NL2010/050716, issued April 26, 2012.	☐

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Signature	/Ashley Hamlett/	Date (YYYY-MM-DD)	2019-08-14
Name/Print	Ashley Hamlett	Registration Number	62,569

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Examiner Name	
Attorney Docket Number	VONL:004USC1

/R.T.B/	1	BUSO, et al., "Tuber Yield and Quality of 4x-2x (FDR) Potato Progenies Derived from the Wild Diploid Species Solanum Berthaultii and Solanum Tarijense," Plant Breeding, 122, 229-232 (2003).	☐
/R.T.B/	2	DeMAINE, "The Effects of Inbreeding on the Parental Values of Potato Dihaploids," Ann. Appl. Biol. (1995) 127: 151-156.	☐
/R.T.B/	3	EIJLANDER, et al., "Selection of Vigorous and Fertile S-homo- and Heterozygous Tester Clones From Self-Incompatible Diploid Potato, Solanum Tuberosum L.," Euphytica, 97: 97-111, 1997.	☐
/R.T.B/	4	HERMSEN, et al., "Inheritance of Genetic Markers From Two Potato Dihaploids and Their Respective Parent Cultivars," Euphytica, 27 (1978) 681-688.	☐
/R.T.B/	5	HERMUNDSTAD, et al., "Tuber Yield and Tuber Traits of Haploid-Wild Species FI Hybrids," Potato Research 29 (1986) 289-297.	☐
/R.T.B/	6	JANSKY, "Overcoming Hybridization Barriers in Potato," Plant Breeding, 125, 1-12 (2006).	☐
/R.T.B/	7	PABLO, et al., "Crossability Relationships Among the Wild Diploid Potato Species Solanum Kurtzianum, S. Chacoense and S. Ruiz-Lealii from Argentina," Euphytica, 132: 2987-295, 2003.	☐
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Title of Invention:	Hybrid Seed Potato Breeding
First Named Inventor/Applicant Name:	Willem Hendrik Lindhout
Customer Number:	125771
Filer:	Robert E. Hanson/LeRey Coker
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Attorney Docket Number:	VONL:004USC1
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Title of Invention	Hybrid Seed Potato Breeding

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number 16/541,071 filed on August 14, 2019.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

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LEGAL NAME OF INVENTOR

Inventor: Willem Hendrik Lindhout Date (Optional): x Sept 26th 2019

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Inventor: Teunis Philippus Schotte Date (Optional): x

Signature: x

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Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Richard Gerardus Franciscus Visser Date (Optional): x 2/6/2019

Signature: x R.G.F. Visser

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/AIA/01 (06-12)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	Hybrid Seed Potato Breeding

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number 16/541,071 filed on August 14, 2019.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

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LEGAL NAME OF INVENTOR

Inventor: Herman Johannes van Eck Date (Optional): x 20 October 2019

Signature: x

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

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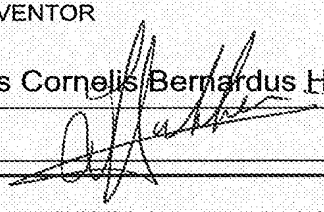
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LEGAL NAME OF INVENTOR

Inventor: Ronaldus Cornelis Bernardus Hutten Date (Optional): x 4 OKT 2019

Signature: x 

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Willem Hendrik Lindhout *et al.*	Group Art Unit: 1663
Serial No.: 16/541,071	Examiner: Russell T. Boggs
Filed: August 14, 2019	Confirmation No.: 4348
For: Hybrid Seed Potato Breeding	Attorney Docket No.: VONL:004USC1

RESPONSE TO THE INFORMATIONAL NOTICE TO APPLICANT

MS MISSING PARTS
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Commissioner:

In response to the Notice Requiring Inventor's Oath or Declaration, dated October 22, 2019, enclosed are:

(a) A Declaration executed by Willem Hendrik Lindhout, Teunis, Philippis Schotte, Richard Gerardus Franciscus Visser, Herman Johannes van Eck, and Ronaldus Cornelis Bernardus Hutten; and

(b) A copy of the Informational Notice Requiring Inventor's Oath or Declaration, dated October 22, 2019.

No fees are believed to be due in connection with the filing of this paper. However, should any fees under 37 C.F.R. §§ 1.16-1.21 be deemed necessary for any reason relating to these materials, the Commissioner is authorized to deduct the appropriate fees from Dentons US LLP's Deposit Account No.: 19-3140/VONL:004USC1.

Respectfully submitted,

/Robert E. Hanson/

Robert E. Hanson
Reg. No. 42,628
Attorney for Applicant

Dentons US LLP
2000 McKinney Avenue, Suite 1900
Dallas, Texas 75201
(214) 259-0931

Date: December 20, 2019

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/541,071	08/14/2019	Willem Hendrik Lindhout	VONL:004USC1

CONFIRMATION NO. 4348

125771
DENTONS US LLP
P.O. Box 061080
Chicago, IL 60606

INFORMAL NOTICE

OC000000112026363

Date Mailed: 10/22/2019

INFORMATIONAL NOTICE TO APPLICANT

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

The item(s) indicated below are also required and should be submitted with any reply to this notice to avoid further processing delays.

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):
  Willem Hendrik Lindhout
  Teunis Philippus Schotte
  Richard Gerardus Franciscus Visser
  Herman Johannes van Eck
  Ronaldus Cornelis Bernardus Hutten

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/mmasfaw/