PTO-1390 (09-11)
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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A SUBMISSION UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
05032-00255	

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/NL2010/050716	2010-10-26	2009-10-26

TITLE OF INVENTION
Hybrid Seed Potato Breeding

APPLICANT(S) FOR DO/EO/US
Willem Hendrik Lindhout, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☑ This is a **FIRST** submission of items concerning a submission under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a submission under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☑ The US has been elected (Article 31).
5. ☑ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☑ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☑ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☑ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☑ A preliminary amendment.
14. ☑ An Application Data Sheet under 37 CFR 1.76.
15. ☐ A substitute specification.
16. ☐ A power of attorney and/or change of address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13*ter*.3 and 37 CFR 1.821- 1.825.
18. ☐ A second copy of the published International Application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).

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This collection of information is required by 37 CFR 1.414 and 1.491-1 .492. The information is required to obtain or retain a b enefit by the public, w hich is to file (and by the USPTO to pro cess) 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 15 minutes to complete, including gathering information, preparing, and submitting the completed 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 FEE S OR COMPLE TED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
	PCT/NL2010/050716	05032-00255

20. Other items or information:
International Search Report and International Report on Patentability.

The following fees have been submitted	CALCULATIONS	PTO USE ONLY
21. ☑ Basic national fee (37 CFR 1.492(a))..... **$380**	$ 380.00	
22. ☑ Examination fee (37 CFR 1.492(c)) If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4)..... **$0** All other situations..... **$250**	$250.00	
23. ☑ Search fee (37 CFR 1.492(b)) If the written opinion of the ISA/US or the International preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4)..... **$0** Search fee (37 CFR 1.445(a)(2)) has been paid on the international application to the USPTO as an International Searching Authority..... **$120** International Search Report prepared by an ISA other than the US and provided to the Office or previously communicated to the US by the IB..... **$490** All other situations..... **$620**	$ 490.00	
TOTAL OF 21, 22 and 23 =	$1,120.00	

☐ Additional fee for specification and drawings filed in paper over 100 sheets (excluding sequence listing in compliance with 37 CFR 1.821(c) or (e) in an electronic medium or computer program listing in an electronic medium) (37 CFR 1.492(j)).
The fee is **$310** for each additional 50 sheets of paper or fraction thereof.

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof (round **up** to a whole number)	RATE	
- 100 =	/50 =		x $**310**	$

Surcharge of **$130.00** for furnishing any of the search fee, examination fee, or the oath or declaration after the date of commencement of the national stage (37 CFR 1.492(h)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$	
Total claims	34 - 20 =	14	x $ **60**	$840.00	
Independent claims	7 - 3 =	4	x **$250**	$1,000.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$450**	$	
			TOTAL OF ABOVE CALCULATIONS =	$2,960.00	
☑ Applicant claims small entity status. See 37 CFR 1.27. Fees above are reduced by ½.					
			SUBTOTAL =	$ 1,480.00	
Processing fee of **$130.00** for furnishing the English translation later than 30 months from the earliest claimed priority date (37 CFR 1.492(i)). +				$	
			TOTAL NATIONAL FEE =	$ 1,480.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
			TOTAL FEES ENCLOSED =	$ 1,480.00	
			Amount to be refunded:	$	
			Amount to be charged	$	

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.

a. ☐ A check in the amount of $ _____ to cover the above fees is enclosed.

b. ☑ Please charge my Deposit Account No. 190733 in the amount of $ 1,480.00 to cover the above fees.

c. ☑ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 190733.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038. The PTO-2038 should only be mailed or faxed to the USPTO. However, when paying the basic national fee, the PTO-2038 may NOT be faxed to the USPTO.

ADVISORY: If filing by EFS-Web, do **NOT** attach the PTO-2038 form as a PDF along with your EFS-Web submission. Please be advised that this is **not** recommended and by doing so your **credit card information may be displayed via PAIR**. To protect your information, it is recommended paying fees online by using the electronic payment method.

NOTE: Where an appropriate time limit under 37 CFR 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the International Application to pending status.

SEND ALL CORRESPONDENCE TO:

/John P. Iwanicki/
SIGNATURE
John P. Iwanicki
NAME
34,628
REGISTRATION NUMBER

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

PTO/SB/14 (11-08)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	05032-00255
	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.)

Applicant Information:

Applicant 1 Remove

Applicant Authority ◉ Inventor ○ Legal Representative under 35 U.S.C. 117 ○ Party of Interest under 35 U.S.C. 118

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[i]	NL

Citizenship under 37 CFR 1.41(b)[i] NL

Mailing Address of Applicant:

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

Applicant 2 Remove

Applicant Authority ◉ Inventor ○ Legal Representative under 35 U.S.C. 117 ○ Party of Interest under 35 U.S.C. 118

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[i]	NL

Citizenship under 37 CFR 1.41(b)[i] NL

Mailing Address of Applicant:

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

Applicant 3 Remove

Applicant Authority ◉ Inventor ○ Legal Representative under 35 U.S.C. 117 ○ Party of Interest under 35 U.S.C. 118

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[i]	NL

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	05032-00255
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

Citizenship under 37 CFR 1.41(b) i	NL			
Mailing Address of Applicant:				
Address 1	Emmalaan 17			
Address 2				
City	Bennekom	**State/Province**		
Postal Code	6721 ET	**Country**i	NL	

Applicant 4 Remove

Applicant Authority (•) Inventor ( ) Legal Representative under 35 U.S.C. 117 ( ) Party of Interest under 35 U.S.C. 118

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**i	NL
Citizenship under 37 CFR 1.41(b) i	NL		

Mailing Address of Applicant:				
Address 1	Eykmanstraat 9			
Address 2				
City	Wageningen	**State/Province**		
Postal Code	6706 JT	**Country**i	NL	

Applicant 5 Remove

Applicant Authority (•) Inventor ( ) Legal Representative under 35 U.S.C. 117 ( ) Party of Interest under 35 U.S.C. 118

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**i	NL
Citizenship under 37 CFR 1.41(b) i	NL		

Mailing Address of Applicant:				
Address 1	De Thijmenstoren 12			
Address 2				
City	Rhenen	**State/Province**		
Postal Code	3912 AT	**Country**i	NL	

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	22910

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	05032-00255
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

Email Address	jiwanicki@bannerwitcoff.com	Add Email	Remove Email

Application Information:

Title of the Invention	Hybrid Seed Potato Breeding		
Attorney Docket Number	05032-00255	**Small Entity Status Claimed**	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Suggested Class (if any)		**Sub Class (if any)**	
Suggested Technology Center (if any)			
Total Number of Drawing Sheets (if any)	11	**Suggested Figure for Publication (if any)**	

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). Enter either 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.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	22910		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this 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(a)(2) or CFR 1.78(a)(4), and need not otherwise be made part of the specification.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	a 371 of international	PCT/NL2010/050716	2010-10-26
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/NL2010/050716	non provisional of	61/254980	2009-10-26

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	05032-00255
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

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

Foreign Priority Information:

This section allows for the applicant to claim benefit of foreign priority and to identify any prior foreign application for which priority is not claimed. 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(a).

Remove

Application Number	Country [i]	Parent Filing Date (YYYY-MM-DD)	Priority Claimed
09013474.3	EP	2009-10-26	(•) Yes ( ) No

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

Assignee Information:

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

Remove

Assignee 1

If the Assignee is an Organization check here. ☒			
Organization Name	Agventure B.V.		
Mailing Address Information:			
Address 1	Burg. Arriensweg 32		
Address 2			
City	Diepenveen	**State/Province**	
Country [i]	NL	Postal Code	7431 BM
Phone Number		Fax Number	
Email Address			

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

Signature:

A signature of the applicant or representative is required in accordance with 37 CFR 1.33 and 10.18. Please see 37 CFR 1.4(d) for the form of the signature.

Signature	/John P. Iwanicki/			Date (YYYY-MM-DD)	2012-04-26
First Name	John P.	Last Name	Iwanicki	Registration Number	34628

PTO/SB/14 (11-08)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	05032-00255
	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.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Willem Hendrik Lindhout, et al.	)	Examiner:
	)	TBA
Serial No.: TBA	)	
	)	Art Unit: TBA
Filed: April 26, 2012	)	
	)	Conf. No.: TBA
Title: HYBRID SEED POTATO BREEDING	)	

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

PRELIMINARY AMENDMENT

Dear Sir:

Preliminary to examination, please enter the following amendments in the above-referenced application.

Amendment to the specification begins on page 2.

Amendments to the claims begin on page 3.

Remarks begin on page 9.

Amendment to the Specification:

On page 1 of the Specification, please add the following new heading and paragraph after the title, "Hybrid Seed Potato Breeding":

RELATED APPLICATION DATA

This application is a National Stage Application under 35 U.S.C. 371 of co-pending PCT application PCT/NL2010/050716 designating the United States and filed October 26, 2010; which claims the benefit of EP patent application number 09013474.3 and filed October 26, 2009 and U.S. provisional patent application number 61/254,980 and filed October 26, 2009 each of which are hereby incorporated by reference in their entireties.

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Amendments to the Claims:

1. (Original) 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. (Currently Amended) 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 trait~~s~~.

3. (Currently Amended) 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, or ~~and~~ cold sweetening resistance.

4. (Currently Amended) 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. (Currently Amended) A plant of a line according to ~~any one of~~ claims 1~~-4~~.

6. (Original) A seed of a plant according to claim 5.

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

8. (Original) 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:

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(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. (Original) The method of claim 8, wherein said steps a-e 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. (Currently Amended) 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, or ~~and~~ cold sweetening resistance.

11. (Currently Amended) 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.

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12. (Currently Amended) 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. (Currently Amended) 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. (Currently Amended) A diploid potato breeding line~~, more preferably an elite line,~~ obtainable by the method of ~~any one of~~ claims ~~8-13~~.

15. (Original) 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. (Original) 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. (Currently Amended) 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~~.

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18. (Currently Amended) 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 being ~~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, or ~~and~~ cold sweetening resistance.

19. (Currently Amended) 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. (Currently Amended) The method of ~~any one of~~ claim~~s~~ 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. (Original) 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. (Currently Amended) A seed produced by the method of ~~any one of~~ claim~~s~~ 16~~-20~~.

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

24. (Original) Pollen or an ovule of the plant of claim 23.

25. (Original) A tuber from the plant of claim 23.

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

27. (Currently Amended) A tissue culture of the plant of claim 23 ~~or 26~~.

28. (Currently Amended) 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. (Currently Amended) 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. (Currently Amended) A method of producing a hybrid potato plant, said method comprising producing a seed according to a method of ~~any one of~~ claim~~s~~ 16~~-20 or 29~~ and growing said seed into a potato plant.

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

32. (Currently Amended) 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. (Original) 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:

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(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. (Original) 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.

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REMARKS

Prior to examination, Applicant respectfully requests entry of the above amendment. The Commissioner is hereby authorized to charge any additional fees or credit overpayment to Deposit Account No. 19-0733.

Respectfully submitted,

Date: April 26, 2012

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INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2010/050716

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01H1/02 A01H5/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, EMBL, WPI Data, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARIS B: "COMPARISON OF DIPLOID AND TETRAPLOID POTATO FAMILIES DERIVED FROM SOLANUM-PHUREJA X DIHAPLOID SOLANUM-TUBEROSUM HYBRIDS AND THEIR VEGETATIVELY DOUBLED COUNTERPARTS", EUPHYTICA, KLUWER ACADEMIC PUBLISHERS, NL, vol. 46, no. 1, 1 March 1990 (1990-03-01), pages 15-33, XP008132658, ISSN: 0014-2336, DOI: DOI:10.1007/BF00057615 table 8 ----- -/--	1-7,14, 15, 22-28, 31-33

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 March 2011	08/06/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bucka, Alexander

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL2010/050716

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-7, 32, 33(completely); 14, 15, 21-28, 31(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SHELLEY HERMUNDSTAD ET AL: "Tuber yield and tuber traits of haploid-wild species F1 hybrids", POTATO RESEARCH, ASSOCIATION FOR POTATO RESEARCH, WAGENINGEN, NL, vol. 29, 1 September 1986 (1986-09-01), pages 289-297, XP008132723, ISSN: 0014-3065, DOI: DOI:10.1007/BF02359958 page 290; figure 1	1-7,14, 15, 22-28, 31-33
X	WATANABE K N ET AL: "Generation of pest resistant, diploid potato germplasm with short-day adaptation from diverse genetic stocks", BREEDING SCIENCE, vol. 46, no. 4, 1996, pages 329-336, XP002577156, tables 1,2,8	1-7,14, 15, 22-28, 31-33
X	WANG D ET AL: "Improving main agronomic characters of dihaploids with 2n gametes by means of sexual hybridisation and anther culture of potato (Solanum tuberosum)", NEW ZEALAND JOURNAL OF CROP AND HORTICULTURAL SCIENCE, vol. 28, no. 1, March 2000 (2000-03), pages 1-8, XP002626196, ISSN: 0114-0671 table 3	1-7,14, 15, 22-28, 31-33
X	PHUMICHAI CHALERMPOL ET AL: "Toward the development of highly homozygous diploid potato lines using the self-compatibility controlling Sli gene", GENOME, vol. 48, no. 6, December 2005 (2005-12), pages 977-984, XP002577501, ISSN: 0831-2796	21
A	figure 1; table 1	1-7,14, 15, 22-28, 31-33

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BANI-AAMEUR F ET AL: "Enhancement of diploid Solanum chacoense Bitt. using adapted clones of Solanum phureja Juz. and Buk", EUPHYTICA, KLUWER ACADEMIC PUBLISHERS, NL, vol. 68, no. 3, 1 January 1993 (1993-01-01), pages 169-179, XP008132638, ISSN: 0014-2336, DOI: DOI:10.1007/BF00029870 figure 4	1-7,14, 15, 21-28, 31-33
A	EP 0 913 081 A1 (PRESIDENT OF KOBE UNIVERSITY A [JP]) 6 May 1999 (1999-05-06) page 6	1-7,14, 15, 21-28, 31-33
A	BUSO J A 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, vol. 122, no. 3, June 2003 (2003-06), pages 229-232, XP002626126, ISSN: 0179-9541 table 2	1-7,14, 15, 21-28, 31-33
A	VISKER M H P W ET AL: "Correlation between late blight resistance and foliage maturity type in potato", EUPHYTICA, vol. 137, no. 3, June 2004 (2004-06), pages 311-323, XP002577157, ISSN: 0014-2336 tables 1,2	1-7,14, 15, 21-28, 31-33
A	DINU I I ET AL: "Novel inter-series hybrids in Solanum, section Petota", THEORETICAL AND APPLIED GENETICS ; INTERNATIONAL JOURNAL OF PLANT BREEDING RESEARCH, SPRINGER, BERLIN, DE LNKD- DOI:10.1007/S00122-004-1782-X, vol. 110, no. 3, 1 February 2005 (2005-02-01), pages 403-415, XP019321811, ISSN: 1432-2242 page 409, column 410; table 2	1-7,14, 15, 21-28, 31-33

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JUAN PABLO RAIMONDI ET AL: "Crossability relationships among the wild diploid potato species Solanum kurtzianum, S. chacoense and S. ruiz-lealii from Argentina.", EUPHYTICA, vol. 132, no. 3, 2003, pages 287-295, XP002577155, ISSN: 0014-2336 tables 1,2,3	1-7,14, 15, 21-28, 31-33
A	EIJLANDER R ET AL: "Selection of vigorous and fertile S-homo- and heterozygous tester clones from self-incompatible diploid potato, Solanum tuberosum L", EUPHYTICA, vol. 97, no. 1, 1997, pages 97-111, XP002577160, ISSN: 0014-2336 table 1	1-7,14, 15, 21-28, 31-33
A	HOSAKA KAZUYOSHI ET AL: "Genetics of self-compatibility in a self-incompatible wild diploid potato species Solanum chacoense. 1. Detection of an S locus inhibitor (Sli) gene", EUPHYTICA, vol. 99, no. 3, 1998, pages 191-197, XP002577158, ISSN: 0014-2336 the whole document	1-7,14, 15, 21-28, 31-33
A	HERMSEN J G T ET AL: "INHERITANCE OF GENETIC MARKERS FROM 2 POTATO DI HA PLOIDS AND THEIR RESPECTIVE PARENT CULTIVARS", EUPHYTICA, KLUWER ACADEMIC PUBLISHERS, NL, vol. 27, no. 3, 1 January 1978 (1978-01-01), pages 681-688, XP008131098, ISSN: 0014-2336 page 681 - page 682; table 1	1-7,14, 15, 21-28, 31-33
A	DE MAINE M J: "The effects of inbreeding on the parental values of potato dihaploids", ANNALS OF APPLIED BIOLOGY, vol. 127, no. 1, 1995, pages 151-156, XP002626197, ISSN: 0003-4746 tables 1,2	1-7,14, 15, 21-28, 31-33

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JANSKY S: "Overcoming hybridization barriers in potato", PLANT BREEDING, vol. 125, no. 1, February 2006 (2006-02), pages 1-12, XP002626127, ISSN: 0179-9541 the whole document	1-7,14, 15, 21-28, 31-33
A	SIMMONDS N W: "A review of potato propagation by means of seed, as distinct from clonal propagation by tubers", POTATO RESEARCH, vol. 40, 1997, pages 191-214, XP002575879, ISSN: 0014-3065 the whole document	1-7,14, 15, 21-28, 31-33

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/NL2010/050716

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0913081 A1	06-05-1999	CA 2252304 A1	30-04-1999
		DE 69810423 D1	06-02-2003
		DE 69810423 T2	02-10-2003
		JP 11127712 A	18-05-1999
		US 6084151 A	04-07-2000

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-7, 32, 33(completely); 14, 15, 21-28, 31(partially)

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.

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2. claims: 8-13, 29(completely); 14, 15, 21-28, 30, 31(partially)

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.

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3. claims: 16(completely); 17-20, 22-28, 30, 31(partially)

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%

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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; plants produced by this method.

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4. claim: 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.

PATENT COOPERATION TREATY

REGISTERED

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Vereenigde
Johan De Wittlaan 7
2517 JR Den Haag
PAYS-BAS

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	13.02.2012

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
P87313PC00	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL2010/050716	26.10.2010	26.10.2009

Applicant
Agventure B.V.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:	Authorized Officer
European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Fax: +31 70 340 - 3016	van Rooijen, Babara Tel. +31 70 340-4567

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P87313PC00	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/NL2010/050716	International filing date *(day/month/year)* 26.10.2010	Priority date *(day/month/year)* 26.10.2009

International Patent Classification (IPC) or national classification and IPC
INV. A01H1/02

Applicant
Agventure B.V.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *(sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and/or sheets containing rectifications authorized by this Authority, unless those sheets were superseded or cancelled, and any accompanying letters (see Rules 46.5, 66.8, 70.16, 91.2, and Section 607 of the Administrative Instructions).
      ☐ sheets containing rectifications, where the decision was made by this Authority not to take them into account because they were not authorized by or notified to this Authority at the time when this Authority began to draw up this report, and any accompanying letters (Rules 66.4bis, 70.2(e), 70.16 and 91.2).
      ☐ superseded sheets and any accompanying letters, where this Authority either considers that the superseding sheets contain an amendment that goes beyond the disclosure in the international application as filed, or the superseding sheets were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in item 4 of Box No. I and the Supplemental Box (see Rule 70.16(b)).
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see paragraph 3*bis* of Annex C of the Administrative Instructions).

4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08.09.2011	13.02.2012

Name and mailing address of the international preliminary examining authority:
European Patent Office P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040
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Authorized officer
Bucka, Alexander
Telephone No. +31 70 340-2279

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a) and (b))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-67 as originally filed

   Claims, Numbers

   1-34 as originally filed

   Drawings, Sheets

   1/10-10/10 as originally filed

   ☒ a sequence listing - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since either they are considered to go beyond the disclosure as filed, or they were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in the Supplemental Box (Rules 70.2(c) and (c-bis)):
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:

5. ☐ This report has been established:
   - ☐ taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91 (Rules 66.1(d-bis) and 70.2(e)).
   - ☐ without taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91(Rules 66.4bis and 70.2(e)).

6. ☐ Supplementary international search report(s) from Authority(ies) has/have been received and taken into account in establishing this report (Rule 45bis.8(b) and (c)).

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 8-13, 16-20, 29, 30, 34(completely); 14, 15, 21-28, 31(partially)

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*.

☒ no international search report has been established for the said claims Nos. 8-13, 16-20, 29, 30, 34(completely); 14, 15, 21-28, 31(partially)

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b) and 13*ter*.2.

☒ See separate sheet for further details

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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-7, 14, 15, 21-28, 31-33
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-7, 14, 15, 21-28, 31-33
Industrial applicability (IA)	Yes:	Claims	1-7, 14, 15, 21-28, 31-33
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

   ☒ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search and/or examination

   ☐ to this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the sequence listing, which forms part of the basis of the report, may be marked "superseded."*

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

According to Rule 66.1(e) PCT, claims relating to inventions, in respect of which no international search report has been established, need not be the subject of international preliminary examination. Therefore, no Written Opinion of the International Searching Authority has been established for the subject matter of present claims 8-13, 16-20, 29, 30, 34 (completely), 14, 15, 21-28, 31 (partially), relating to inventions 2 to 4, which have not been searched due to a lack of unity of invention. The PCT International Search and Preliminary Examination Guidelines, 10.72, state that if "the applicant has not availed himself of the opportunity to have the international search report issued on at least some of the other inventions, this must be taken as an indication that the applicant is prepared for the international application to proceed on the basis that it relates to the invention first mentioned in the claims as originally contained in the international application as filed".

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Reference is made to the following documents:

D1 MARIS B: "COMPARISON OF DIPLOID AND TETRAPLOID POTATO FAMILIES DERIVED FROM SOLANUM-PHUREJA X DIHAPLOID SOLANUM-TUBEROSUM HYBRIDS AND THEIR VEGETATIVELY DOUBLED COUNTERPARTS", EUPHYTICA, KLUWER ACADEMIC PUBLISHERS, NL, vol. 46, no. 1, 1 March 1990 (1990-03-01), pages 15-33, XP008132658, ISSN: 0014-2336, DOI: DOI:10.1007/BF00057615

D2 SHELLEY HERMUNDSTAD ET AL: "Tuber yield and tuber traits of haploid-wild species F1 hybrids", POTATO RESEARCH, ASSOCIATION FOR POTATO RESEARCH, WAGENINGEN, NL, vol. 29, 1 September 1986 (1986-09-01), pages 289-297, XP008132723, ISSN: 0014-3065, DOI: DOI:10.1007/BF02359958

D3 WATANABE K N ET AL: "Generation of pest resistant, diploid potato germplasm with short-day adaptation from diverse genetic stocks", BREEDING SCIENCE, vol. 46, no. 4, 1996, pages 329-336, XP002577156,

D4 WANG D ET AL: "Improving main agronomic characters of dihaploids with 2n gametes by means of sexual hybridisation and anther culture of potato (Solanum tuberosum)", NEW ZEALAND JOURNAL OF CROP AND HORTICULTURAL SCIENCE, vol. 28, no. 1, March 2000 (2000-03), pages 1-8, XP002626196, ISSN: 0114-0671

D5 PHUMICHAI CHALERMPOL ET AL: "Toward the development of highly homozygous diploid potato lines using the self-compatibility controlling Sli gene", GENOME, vol. 48, no. 6, December 2005 (2005-12), pages 977-984, XP002577501, ISSN: 0831-2796

2 The present application appears to meet the criteria of Article 33(1) PCT, because the subject matter of **claims 1 to 7, 14, 15, 21 to 28 and 31 to 33** is new within the meaning of Article 33(2) PCT. While the prior art describes essentially homozygous, diploid, fertile potato plants with tuber yields of at least 200 g per plant (cf. D1, table 8; D2, page 290, "Results", figure 1; D3, table 8; D4, table 3), the cited documents do not test or characterise the self-compatibility of the described plants and therefore the self-compatible, diploid potato plants of claim 1 do not seem to be anticipated by the prior art.

3 D3, which could be regarded as the most relevant prior art for the subject matter of **claim 1**, describes essentially homozygous, diploid, fertile potato plants with tuber yields of at least 200 g per plant, from which the subject matter of claim 1 differs in that it requires the plants to be self-compatible.

The Applicant's attention is drawn to the fact that the claims do not require that the yield is obtained from potato plants grown from seedlings; hence, the plants described in the prior art meet the requirement of an average tuber yield of 200 grams per plant recited in claim 1. The plants of the prior art are likewise considered to be "essentially homozygous", which is defined as being at least 25% homozygous, when testing 100 loci (cf. description, page 21), i. e. the plants need only be homozygous in 25 loci.

The Applicant has contested that the plants described in D3 would meet the requirements of being "essentially homozygous" within the meaning of the description, since this required, according to the Applicant, the testing of 100 loci chosen *randomly* over the whole genome. However, the description, page 21, defines the term "essentially homozygous" as "a level of homozygosity of at least 25% ... homozygosity when testing 100, preferably 1000, more preferably at least 10.000 loci". The description does not require that these loci are chosen randomly. Therefore, the Applicant's argument concerning the homozygosity cannot convince.

Hence, in view of D3, the problem to be solved is the provision of further diploid, essentially homozygous, fertile potato plants with tuber yields of at least 200 g per plant.

The solution proposed by the subject matter of claim 1 consists of plants that are self-compatible.

The Applicant submitted that the problem was "to provide a diploid potato plant that is self-compatible and essentially homozygous and has a high tuber yield also from seedling plants". As already mentioned, the claims do not refer to yield from seedling plants. Since the plants of D3 have a yield of more than 200 g per plant (i. e. up to 600 g per plant; cf. D3, table 8), the yield recited in claim 1 is not a feature differing from the plants of the closest prior art. The same applies to the homozygosity, which is defined by the application as being homozygous in 25 loci (*supra*). Therefore, the only difference to the plants of D3 is their self-compatibility.

The advantages of self-compatible potato plants were *inter alia* known from D5 (cf. D5, page 978, left column), the teaching of which the skilled person would have been aware of, since it is a document from the same technical field. Starting from D3 and having regard of the teaching of D5, the skilled person would have considered the provision of self-compatible potato lines (cf. D5, the whole document). While D5 does not disclose the yield of the self-compatible potato plants, it appears to be self-evident that a breeder is interested in increasing the yield of any given crop. Therefore, the solution proposed by the present application cannot be considered to be inventive.

The same objections apply *mutatis mutandis* to the subject matter of **claims 2 to 7, 14, 15, 22 to 28 and 31 to 33**.

As far as the homozygosity is concerned, it is of course evident that once self-compatible lines are obtained (by following the teaching of D5), an increase in their homozygosity is a mere routine and readily achievable (cf. D5, table 2).

The Applicant has furthermore argued that there was a prejudice against breeding homozygous breeding lines, relying on a document from 1987 and an internet disclosure, both of which were however not submitted. Consequently, it is impossible to assess their teaching. However, a prejudice in the art is usually defined as an opinion widely or universally held by experts in the field, normally demonstrated by reference to reviews or encyclopedias. It appears that the existence of such a prejudice has not been sufficiently proven in the present case.

4 Claim 21 relates to deposited diploid, fertile, self-compatible potato plants with different degrees of homozygosity. Their yield is characterised as intermediate/high, although exact data are not disclosed in the application.

Document D5, which is considered to represent the most relevant state of the art for the subject matter of claim 21, discloses (cf. D5, figure 1, table 1) diploid, fertile, self-compatible, homozygous potato plants (inbred series B), from which the subject matter of claim 21 differs in that provides apparently genetically different diploid potato plants.

The problem to be solved by the present invention may therefore be regarded as the provision of further diploid potato plants.

The solution proposed in **claim 21** of the present application cannot be considered as involving an inventive step (Article 33(3) PCT), since the skilled person obviously would have considered the provision of further diploid homozygous self-compatible potato lines based e. g. on the teaching of document D5 (cf. D5, figure 1, table 1). It is emphasized that the yield of the deposited lines is not quantitated and that some lines are only 25% homozygous. Hence, the Applicant's arguments concerning the yield and the homozygosity, submitted in reply to the Written Opinion of the International Searching Authority, do not appear to apply to (some of) the deposited lines. The deposited lines do not appear to be associated with any unexpected or surprising technical effect.

5 According to the PCT Guidelines, A5.26[1], claims for products defined in terms of a process of manufacture (cf. **claims 14, 15, 22 to 28, 31, 33**) are to be construed as claims to the product as such. A product is not rendered novel merely by the fact that it is produced by means of a new process.

6 The attention of the Applicant is drawn to the fact that the application may not be amended in such a way that it contains subject matter which extends beyond the content of the application as filed (Article 34(2)(b) PCT).

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Page 38

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:	John P. Iwanicki/Graci Smith
Attorney Docket Number:	05032-00255

Filed as Small Entity

U.S. National Stage under 35 USC 371 Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Basic National Stage Fee	2631	1	190	190
Natl Stage Search Fee - Report provided	2642	1	245	245
Natl Stage Exam Fee - all other cases	2633	1	125	125
Pages:				
Claims:				
Claims in excess of 20	2615	14	30	420
Independent claims in excess of 3	2614	4	125	500
Miscellaneous-Filing:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**1480**

Electronic Acknowledgement Receipt

EFS ID:	12633708
Application Number:	13504156
International Application Number:	PCT/NL10/50716
Confirmation Number:	6337
Title of Invention:	Hybrid Seed Potato Breeding
First Named Inventor/Applicant Name:	Willem Hendrik Lindhout
Customer Number:	22910
Filer:	John P. Iwanicki/Graci Smith
Filer Authorized By:	John P. Iwanicki
Attorney Docket Number:	05032-00255
Receipt Date:	26-APR-2012
Filing Date:	
Time Stamp:	10:46:37
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$1480
RAM confirmation Number	12501
Deposit Account	190733
Authorized User	

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

Charge any Additional Fees required under 37 C.F.R. 1.492 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal of New Application	Transmittal.pdf	246642 5f3130d7287d16a2207faba6edd0cf2f1ba7893d	no	4
Warnings:					
Information:					
2	Application Data Sheet	ADS.pdf	1421225 fc7675849fdb4e47b2f74b45d9747e5979e90396	no	6
Warnings:					
Information:					
3		PreliminaryAmendment.pdf	85421 992f4039a7d1b0ac7e18828899363 68b99bbd19d	yes	9

Multipart Description/PDF files in .zip description

Document Description	Start	End
Preliminary Amendment	1	1
Specification	2	2
Claims	3	8
Applicant Arguments/Remarks Made in an Amendment	9	9

Warnings:					
Information:					
4	Documents submitted with 371 Applications	ISR.pdf	7577758 d4435865faa54157ab03648e4336103029dad0a7	no	9
Warnings:					
Information:					
5	Documents submitted with 371 Applications	IPRP.pdf	1267181 aaa707ac691f795bec26d04c180012b80064a446	no	10
Warnings:					
Information:					
6	Fee Worksheet (SB06)	fee-info.pdf	38052 e1762a5867f592ccce3d73cc6087de0860afe0a6	no	2

Warnings:	
Information:	
Total Files Size (in bytes):	10636279

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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.

Electronic Acknowledgement Receipt

EFS ID:	12633708
Application Number:	13504156
International Application Number:	PCT/NL10/50716
Confirmation Number:	6337
Title of Invention:	Hybrid Seed Potato Breeding
First Named Inventor/Applicant Name:	Willem Hendrik Lindhout
Customer Number:	22910
Filer:	John P. Iwanicki/Graci Smith
Filer Authorized By:	John P. Iwanicki
Attorney Docket Number:	05032-00255
Receipt Date:	26-APR-2012
Filing Date:	
Time Stamp:	10:46:37
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
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Payment was successfully received in RAM	$1480
RAM confirmation Number	12501
Deposit Account	190733
Authorized User	

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

Charge any Additional Fees required under 37 C.F.R. 1.492 (National application filing, search, and examination fees)

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Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal of New Application	Transmittal.pdf	246642 5f3130d7287d16a2207faba6edd0cf2f1ba7893d	no	4
Warnings:					
Information:					
2	Application Data Sheet	ADS.pdf	1421225 fc7675849fdb4e47b2f74b45d9747e5979e90396	no	6
Warnings:					
Information:					
3		PreliminaryAmendment.pdf	85421 992f4039a7d1b0ac7e18828899363 68b99bbd19d	yes	9

Multipart Description/PDF files in .zip description

Document Description	Start	End
Preliminary Amendment	1	1
Specification	2	2
Claims	3	8
Applicant Arguments/Remarks Made in an Amendment	9	9

Warnings:					
Information:					
4	Documents submitted with 371 Applications	ISR.pdf	7577758 d4435865faa54157ab03648e4336103029dad0a7	no	9
Warnings:					
Information:					
5	Documents submitted with 371 Applications	IPRP.pdf	1267181 aaa707ac691f795bec26d04c180012b80064a446	no	10
Warnings:					
Information:					
6	Fee Worksheet (SB06)	fee-info.pdf	38052 e1762a5867f592ccce3d73cc6087de0860afe0a6	no	1

Warnings:	
Information:	
Total Files Size (in bytes):	10636279

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
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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.

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(72) Inventors; and

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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:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

— *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))*

(54) Title: HYBRID SEED POTATO BREEDING

(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 preparing such plants and to hybrid seeds obtained by crossing such homozygous potato lines and to potato plants grown from said see.

WO 2011/053135 A2

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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, 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.

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-e 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

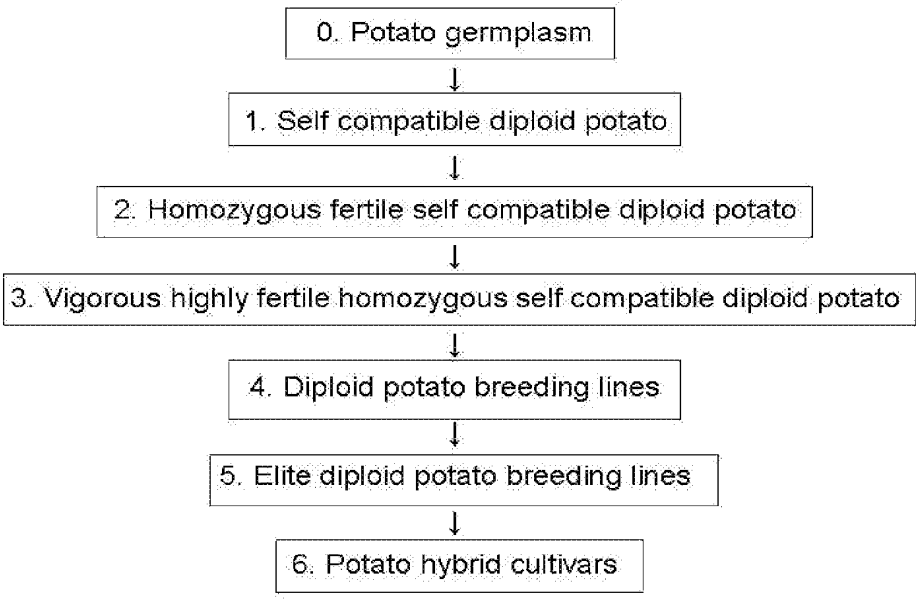
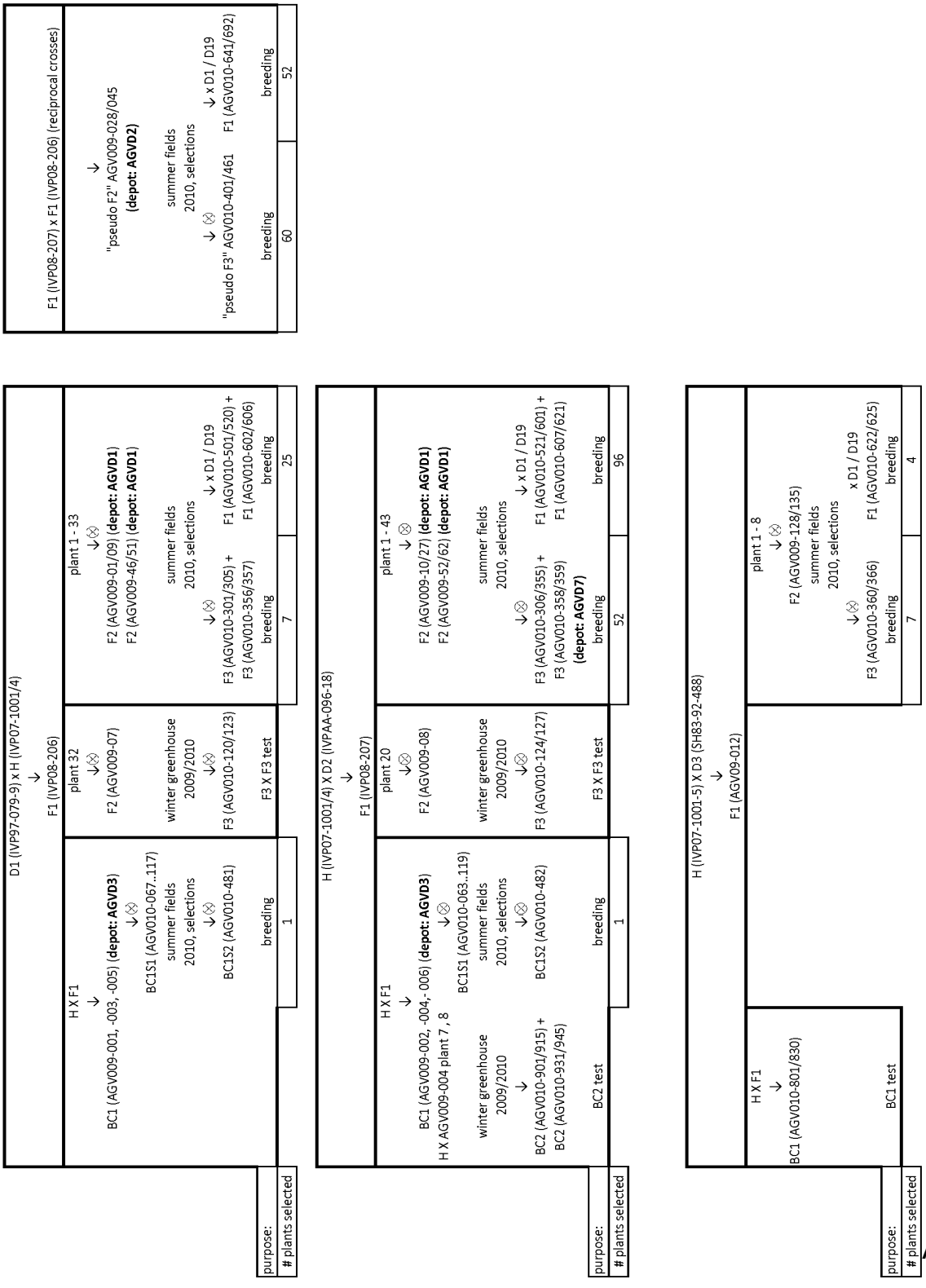

0. Potato germplasm
1. Self compatible diploid potato
2. Homozygous fertile self compatible diploid potato
3. Vigorous highly fertile homozygous self compatible diploid potato
4. Diploid potato breeding lines
5. Elite diploid potato breeding lines
6. Potato hybrid cultivars

Figure 2



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Figure 3

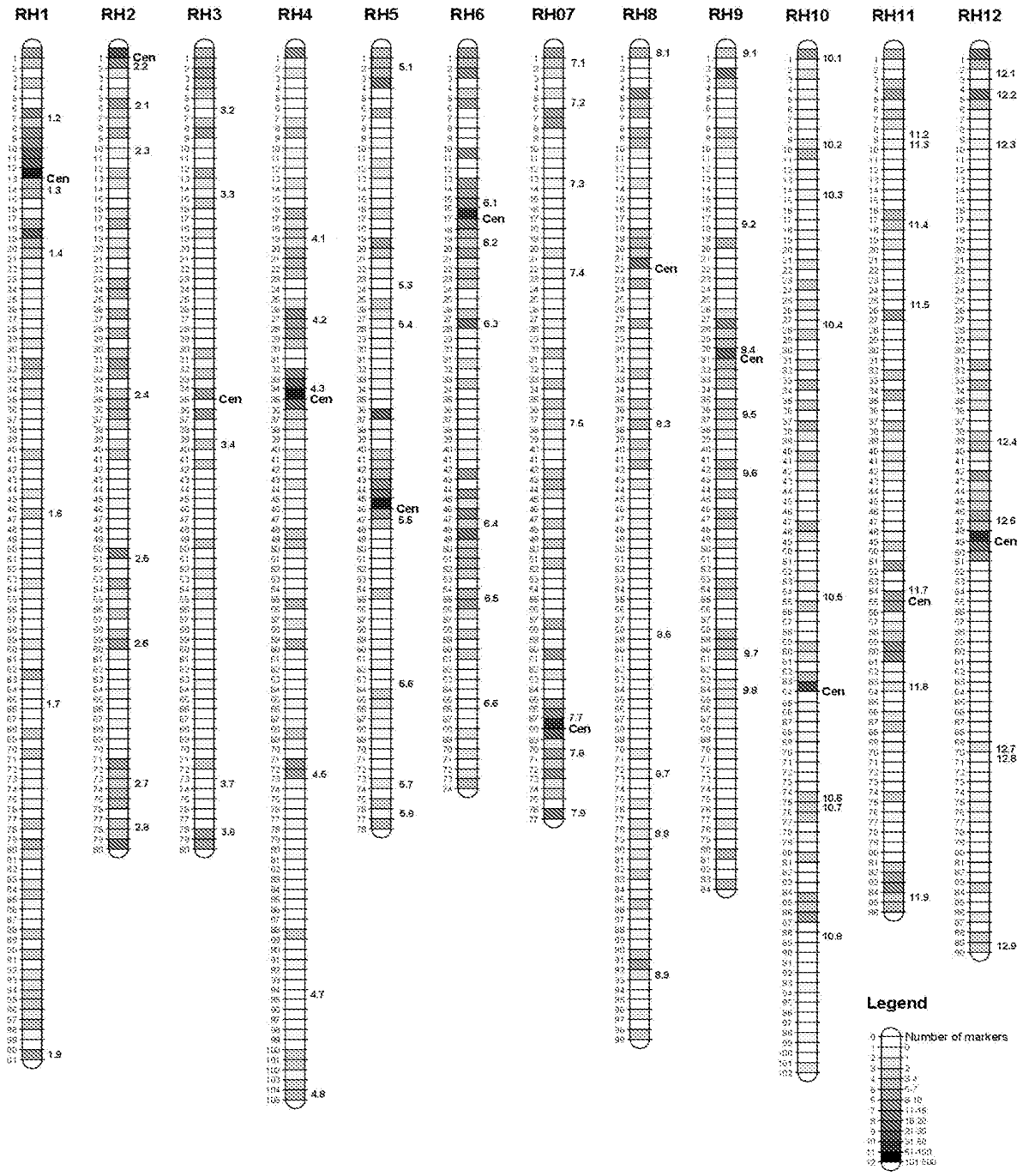
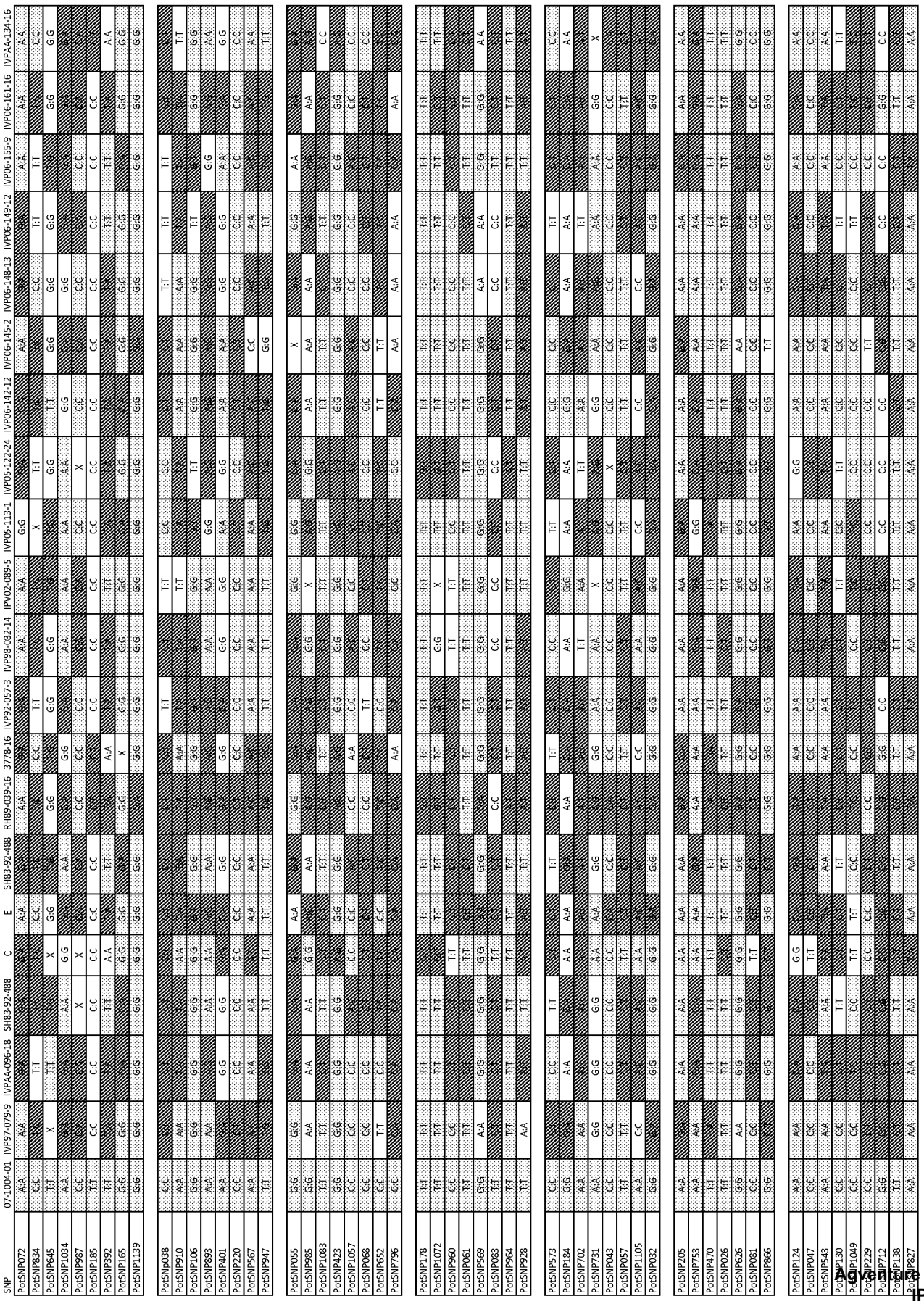

RH1
RH2
RH3
RH4
RH5
RH6
RH07
RH8
RH9
RH10
RH11
RH12
Cen
Legend
Number of markers

Figure 4



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Figure 4 continued

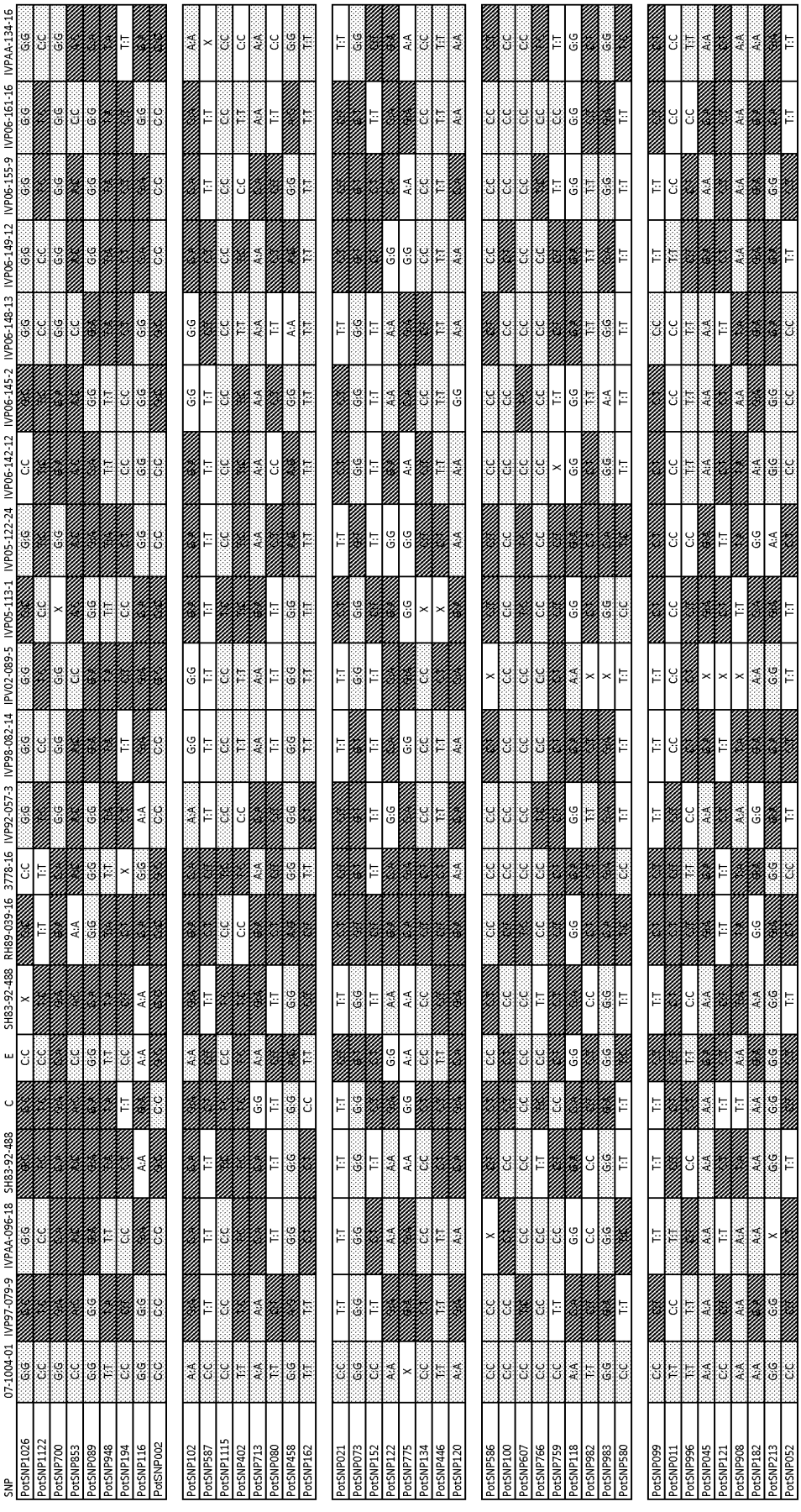


Figure 5 A

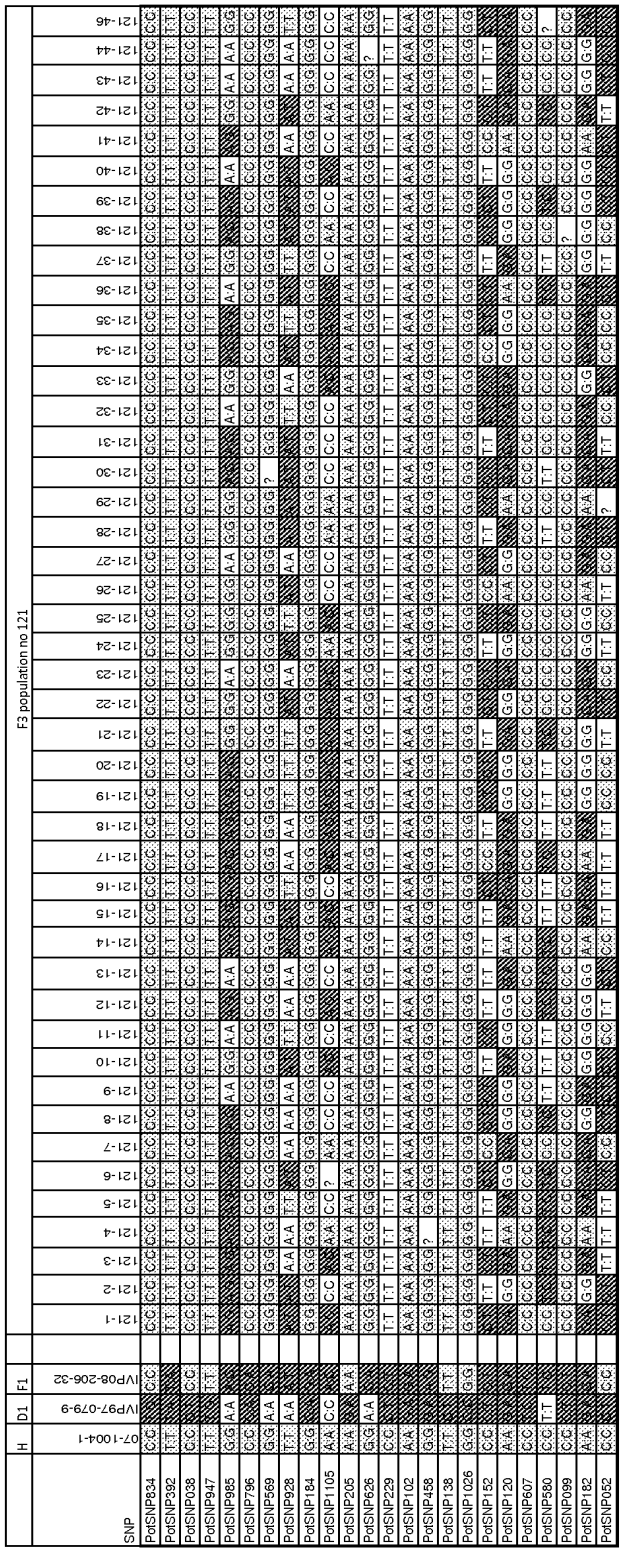

F3 population no 121
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IVP08-206-32
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121-5
121-6
121-7
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121-10
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PotSNP205
PotSNP626
PotSNP229
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PotSNP607
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PotSNP099
PotSNP182
PotSNP052

Figure 5 B

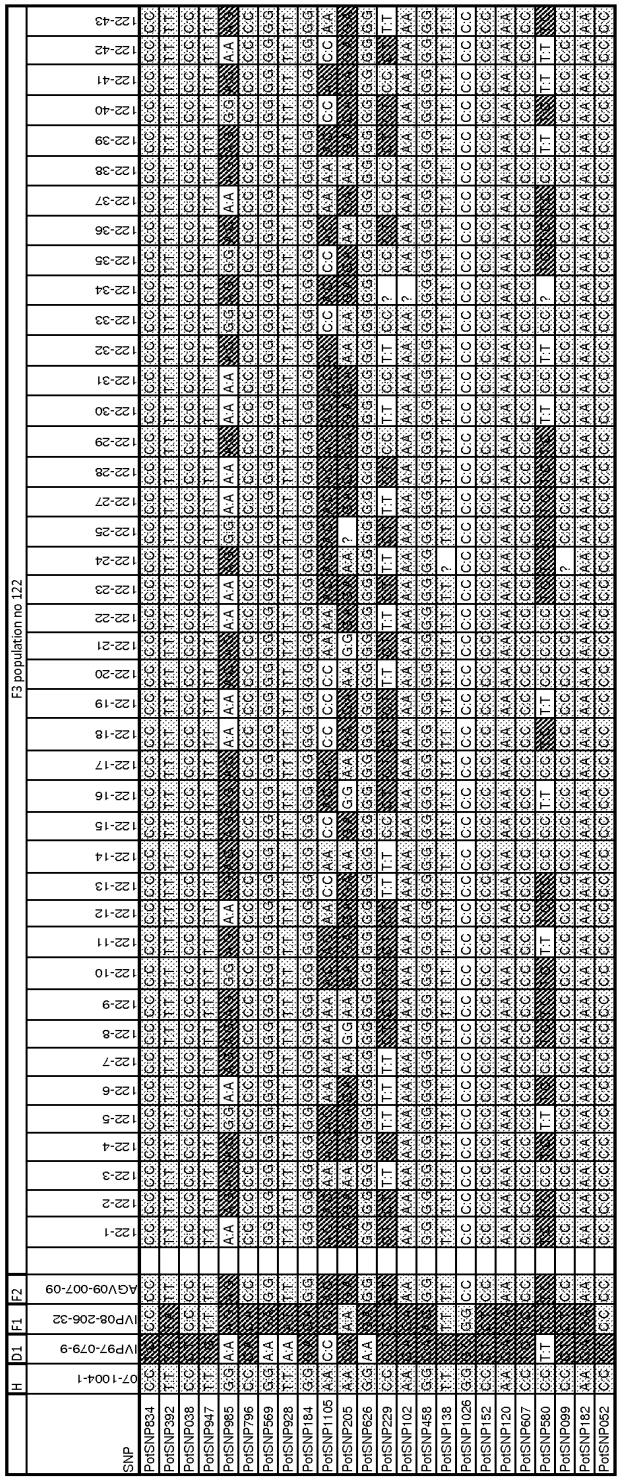

F3 population no 122
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D1
F1
F2
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IVP08-206-32
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PotSNP392
PotSNP038
PotSNP947
PotSNP985
PotSNP796
PotSNP569
PotSNP928
PotSNP184
PotSNP1105
PotSNP205
PotSNP626
PotSNP229
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PotSNP152
PotSNP120
PotSNP607
PotSNP580
PotSNP099
PotSNP182
PotSNP052
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122-43

Figure 5 C

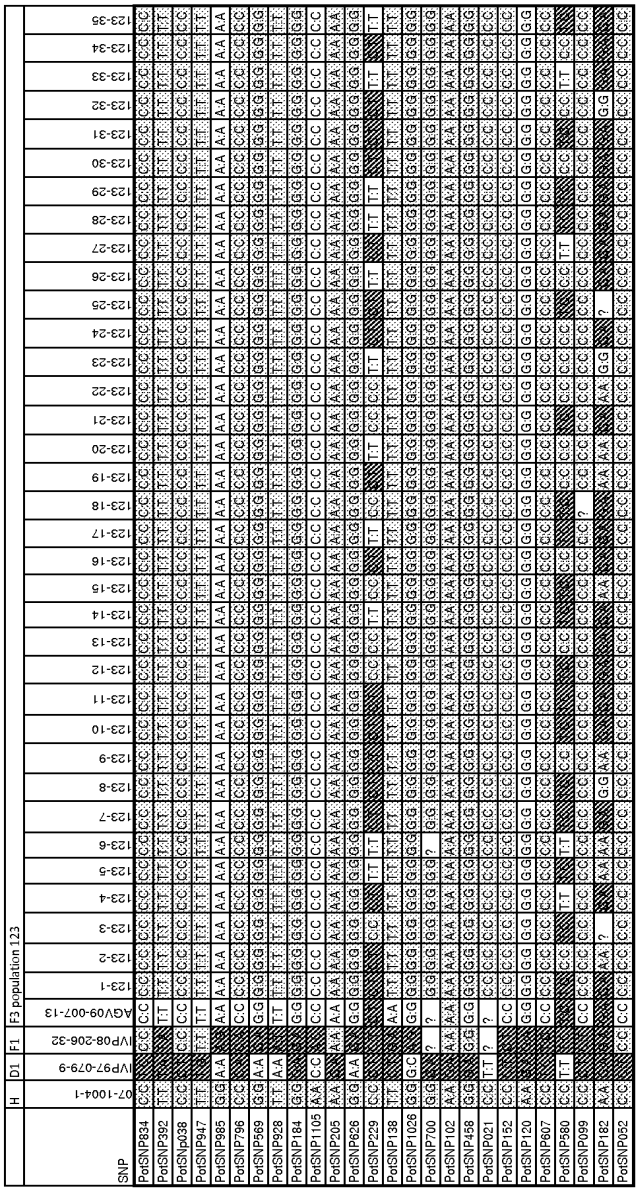

H
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IVP08-206-32
AGV09-007-13
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PotSNP392
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PotSNP947
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Figure 5 D

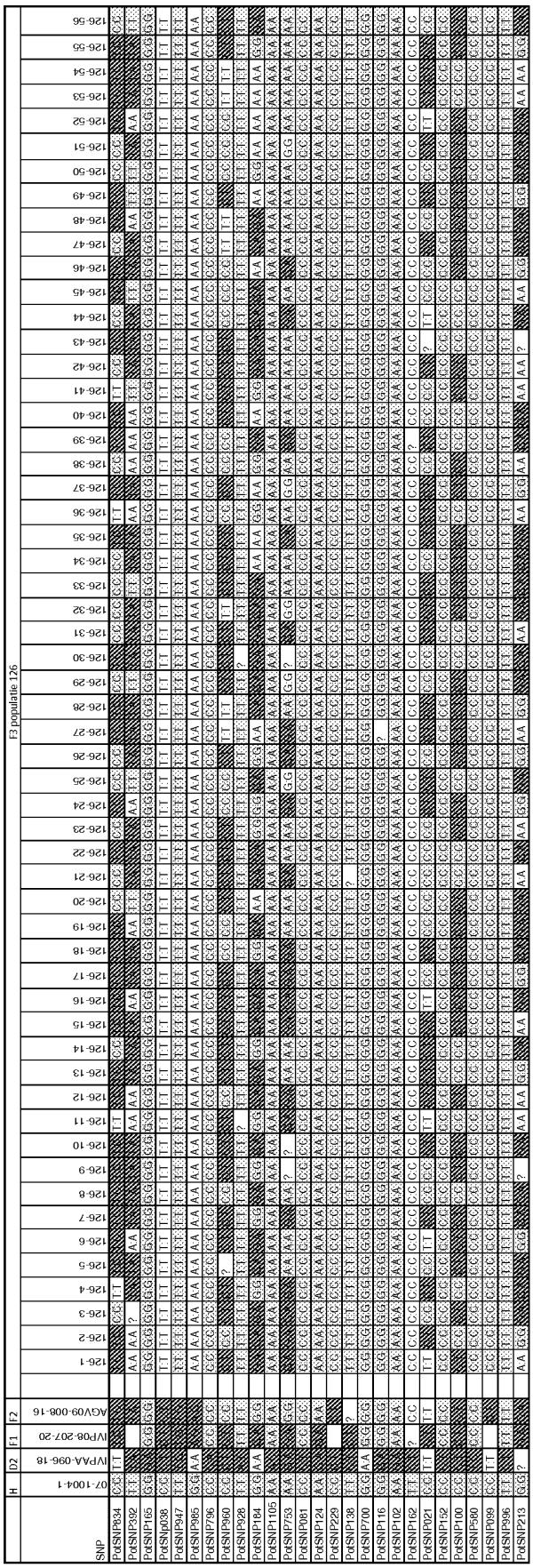

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Figure 5 E

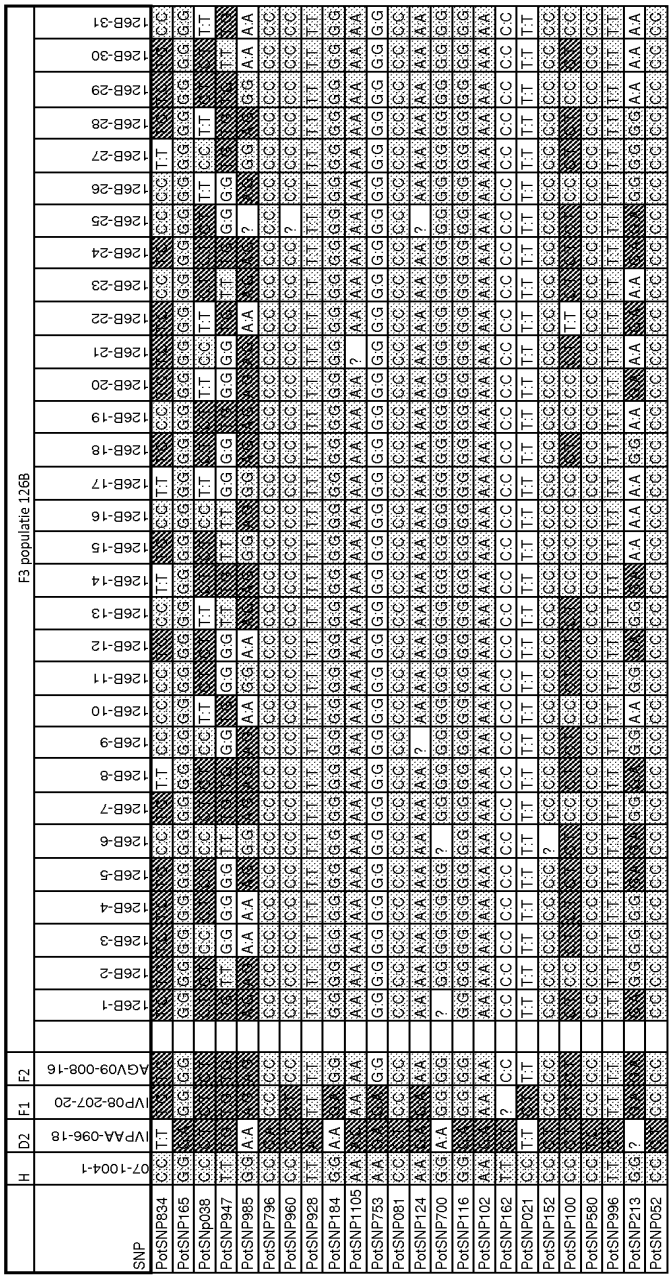
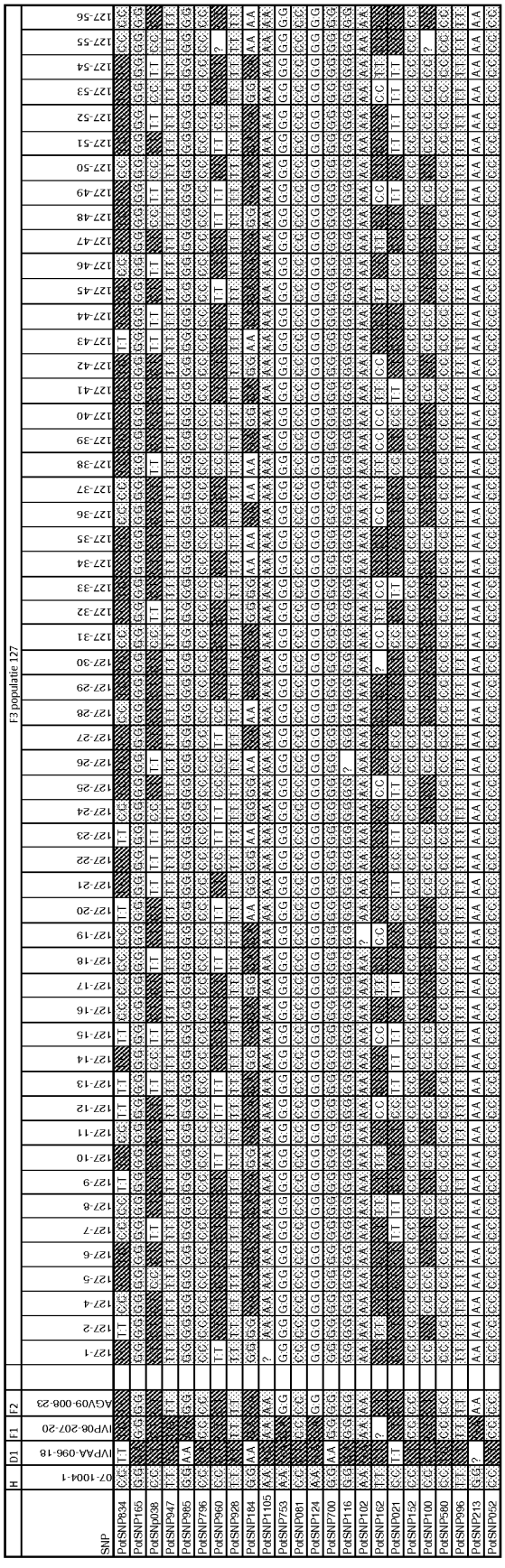

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Figure 5 F


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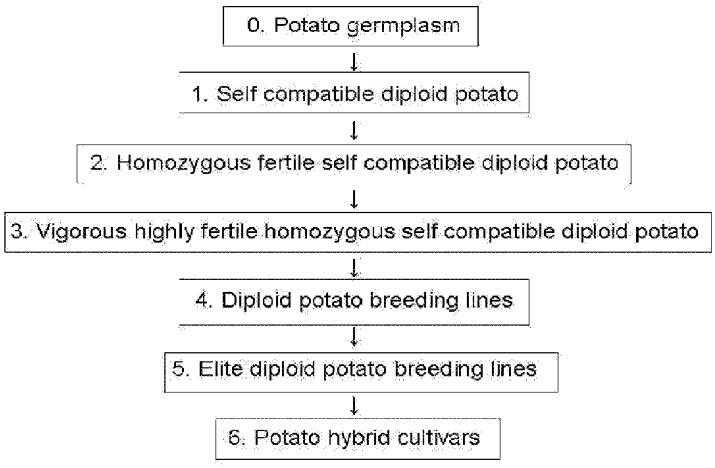
Published:

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[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.

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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

Adventure B.V. Ex. 2009
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Anmelder / Applicant(s) / Demandeur(s):

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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)
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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

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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

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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-

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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

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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

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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).

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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.

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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

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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

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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-

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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*.

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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

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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

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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

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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-

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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

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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

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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-

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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.

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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

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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

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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

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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

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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

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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 recurrent

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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

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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.

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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

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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

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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.

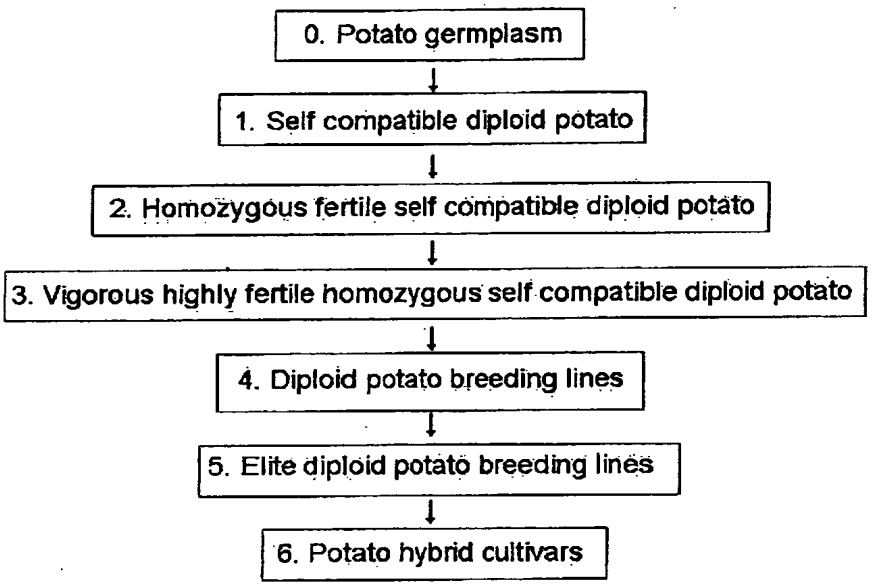
23. A hybrid potato cultivar obtainable by crossing a first and second diploid potato breeding line according to claim 22.

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.

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Figure 1



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Confirmation Number:	1831
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First Named Inventor/Applicant Name:	Willem Hendrik Lindhout
Customer Number:	22910
Filer:	John P. Iwanicki/Michelle DeFalco
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Attorney Docket Number:	005032-00197
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	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Specification		1	29	
	Claims		30	34	
	Abstract		35	35	
Warnings:					
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2	Provisional Cover Sheet (SB16)	005032-00197_Cover_Sheet.pdf	132188 7d5a6508b3a81c88d8b856db88b5784e0ef69a94	no	3
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Provisional Application for Patent Cover Sheet

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Title of Invention	HYBRID SEED POTATO BREEDING
Attorney Docket Number (if applicable)	005032-00197

Correspondence Address

Direct all correspondence to (select one):

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Customer Number	22910

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Entity Status

Applicant claims small entity status under 37 CFR 1.27

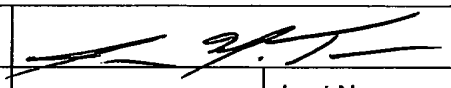
- ○ Yes, applicant qualifies for small entity status under 37 CFR 1.27
- ◉ No

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Signature				Date (YYYY-MM-DD)	2009-10-26
First Name	Jeanne M.	Last Name	Tanner	Registration Number (If appropriate)	45156

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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.

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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

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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.

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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

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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.

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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,

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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.

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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

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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.

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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.

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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 crossing 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:

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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.

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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.

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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

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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.

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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

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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.

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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

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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.

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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 different 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

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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:

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- 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

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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*

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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,

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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.

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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:

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(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.

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Preferably, said step (e2) comprises from 2 to 7 backcrosses using a plant of line AGVD1, AGVD2 or AGVD3 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 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.

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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.

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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.

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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.

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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

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(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

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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.

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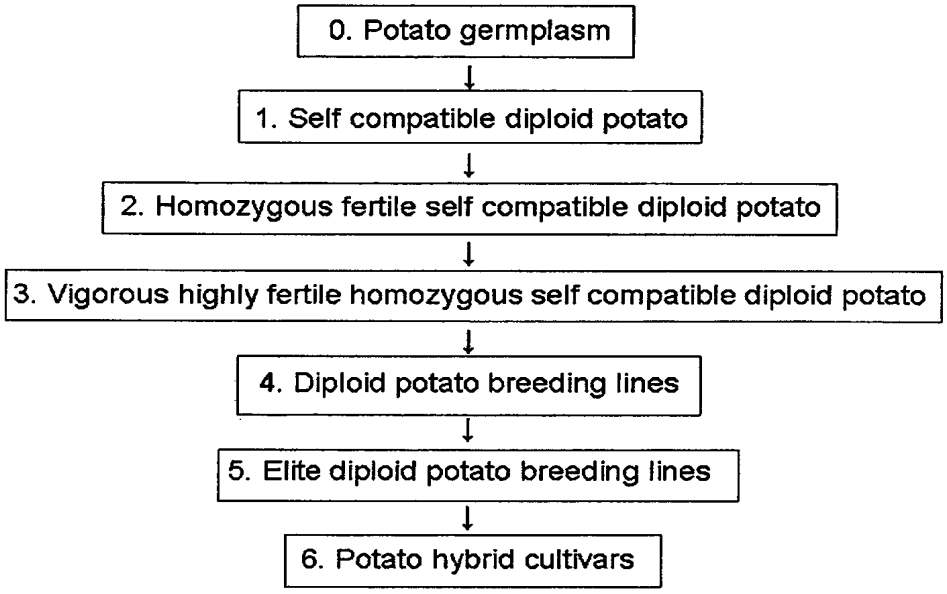
ABSTRACT

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.

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Figure 1



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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	005032-00197
	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.
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Secrecy Order 37 CFR 5.2

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Applicant Information:

Applicant 1

Applicant Authority	◉ Inventor	○ Legal Representative under 35 U.S.C. 117	○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
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Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

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Citizenship under 37 CFR 1.41(b) [i]	NL		

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Address 2			
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Postal Code	6703 BE	Country[i]	NL

Applicant 2

Applicant Authority	◉ Inventor	○ Legal Representative under 35 U.S.C. 117	○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
	Teunis	Phillippus	Schotte	

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Citizenship under 37 CFR 1.41(b) [i]	NL		

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Applicant 3

Applicant Authority	◉ Inventor	○ Legal Representative under 35 U.S.C. 117	○ Party of Interest under 35 U.S.C. 118

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[i]	NL

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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 contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	005032-00197
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

Citizenship under 37 CFR 1.41(b) i	NL		
Mailing Address of Applicant:			
Address 1	Emmalaan 17		
Address 2			
City	Bennekom	**State/Province**	
Postal Code	6721 ET	**Country**i	NL

Applicant 4

Applicant Authority ◉ Inventor	○ Legal Representative under 35 U.S.C. 117	○ Party of Interest under 35 U.S.C. 118	
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**i	NL
Citizenship under 37 CFR 1.41(b) i	NL		
Mailing Address of Applicant:			
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Address 2			
City	Wageningen	**State/Province**	
Postal Code	6706 JT	**Country**i	NL

Applicant 5

Applicant Authority ◉ Inventor	○ Legal Representative under 35 U.S.C. 117	○ Party of Interest under 35 U.S.C. 118	
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**i	NL
Citizenship under 37 CFR 1.41(b) i	NL		
Mailing Address of Applicant:			
Address 1	De Thijmenstoren 12		
Address 2			
City	Rhenen	**State/Province**	
Postal Code	3912 AT	**Country**i	NL

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	22910

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	005032-00197
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

Email Address		Add Email	Remove Email

Application Information:

Title of the Invention	Hybrid Seed Potato Breeding		
Attorney Docket Number	005032-00197	Small Entity Status Claimed ☐	
Application Type	Provisional		
Subject Matter	Utility		
Suggested Class (if any)		Sub Class (if any)	
Suggested Technology Center (if any)			
Total Number of Drawing Sheets (if any)	1	Suggested Figure for Publication (if any)	1

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). Enter either 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.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	22910		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this 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(a)(2) or CFR 1.78(a)(4), and need not otherwise be made part of the specification.

Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)

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

Foreign Priority Information:

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Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	005032-00197
	Application Number	
Title of Invention	Hybrid Seed Potato Breeding	

This section allows for the applicant to claim benefit of foreign priority and to identify any prior foreign application for which priority is not claimed. 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(a).

Remove

Application Number	Country [i]	Parent Filing Date (YYYY-MM-DD)	Priority Claimed
			○ Yes ◉ No

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

Assignee Information:

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

Assignee 1

If the Assignee is an Organization check here. ☒

Organization Name	Agventure B.V.		
Mailing Address Information:			
Address 1	Burg. Arriensweg 32		
Address 2			
City	Diepenveen	**State/Province**	
Country [i] NL		Postal Code	7431 BM
Phone Number		Fax Number	
Email Address			

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

Signature:

A signature of the applicant or representative is required in accordance with 37 CFR 1.33 and 10.18. Please see 37 CFR 1.4(d) for the form of the signature.

Signature				Date (YYYY-MM-DD)	2009-10-26
First Name	Jeanne M.	Last Name	Tanner	Registration Number	45156

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.**

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P87313PC00	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/NL2010/050716	International filing date *(day/month/year)* 26.10.2010	Priority date *(day/month/year)* 26.10.2009
International Patent Classification (IPC) or national classification and IPC INV. A01H1/02		
Applicant Agventure B.V.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *(sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and/or sheets containing rectifications authorized by this Authority, unless those sheets were superseded or cancelled, and any accompanying letters (see Rules 46.5, 66.8, 70.16, 91.2, and Section 607 of the Administrative Instructions).
      ☐ sheets containing rectifications, where the decision was made by this Authority not to take them into account because they were not authorized by or notified to this Authority at the time when this Authority began to draw up this report, and any accompanying letters (Rules 66.4bis, 70.2(e), 70.16 and 91.2).
      ☐ superseded sheets and any accompanying letters, where this Authority either considers that the superseding sheets contain an amendment that goes beyond the disclosure in the international application as filed, or the superseding sheets were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in item 4 of Box No. I and the Supplemental Box (see Rule 70.16(b)).
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see paragraph 3*bis* of Annex C of the Administrative Instructions).

4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08.09.2011	13.02.2012
Name and mailing address of the international preliminary examining authority: European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Fax: +31 70 340 - 3016	Authorized officer Bucka, Alexander Telephone No. +31 70 340-2279

Agventure B.V. Ex. 2009
IPR2025-00136
Page 223

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a) and (b))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-67 as originally filed

   Claims, Numbers

   1-34 as originally filed

   Drawings, Sheets

   1/10-10/10 as originally filed

   ☒ a sequence listing - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since either they are considered to go beyond the disclosure as filed, or they were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in the Supplemental Box (Rules 70.2(c) and (c-bis)):
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:

5. ☐ This report has been established:
   - ☐ taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91 (Rules 66.1(d-bis) and 70.2(e)).
   - ☐ without taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91(Rules 66.4bis and 70.2(e)).

6. ☐ Supplementary international search report(s) from Authority(ies) has/have been received and taken into account in establishing this report (Rule 45bis.8(b) and (c)).

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 8-13, 16-20, 29, 30, 34(completely); 14, 15, 21-28, 31(partially)

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*.

   ☒ no international search report has been established for the said claims Nos. 8-13, 16-20, 29, 30, 34(completely); 14, 15, 21-28, 31(partially)

   ☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

      ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

      ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

      ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b) and 13*ter*.2.

   ☒ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-7, 14, 15, 21-28, 31-33
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-7, 14, 15, 21-28, 31-33
Industrial applicability (IA)	Yes:	Claims	1-7, 14, 15, 21-28, 31-33
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

   ☒ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search and/or examination

   ☐ to this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the sequence listing, which forms part of the basis of the report, may be marked "superseded."*

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

According to Rule 66.1(e) PCT, claims relating to inventions, in respect of which no international search report has been established, need not be the subject of international preliminary examination. Therefore, no Written Opinion of the International Searching Authority has been established for the subject matter of present claims 8-13, 16-20, 29, 30, 34 (completely), 14, 15, 21-28, 31 (partially), relating to inventions 2 to 4, which have not been searched due to a lack of unity of invention. The PCT International Search and Preliminary Examination Guidelines, 10.72, state that if "the applicant has not availed himself of the opportunity to have the international search report issued on at least some of the other inventions, this must be taken as an indication that the applicant is prepared for the international application to proceed on the basis that it relates to the invention first mentioned in the claims as originally contained in the international application as filed".

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Reference is made to the following documents:

D1 MARIS B: "COMPARISON OF DIPLOID AND TETRAPLOID POTATO FAMILIES DERIVED FROM SOLANUM-PHUREJA X DIHAPLOID SOLANUM-TUBEROSUM HYBRIDS AND THEIR VEGETATIVELY DOUBLED COUNTERPARTS", EUPHYTICA, KLUWER ACADEMIC PUBLISHERS, NL, vol. 46, no. 1, 1 March 1990 (1990-03-01), pages 15-33, XP008132658, ISSN: 0014-2336, DOI: DOI:10.1007/BF00057615

D2 SHELLEY HERMUNDSTAD ET AL: "Tuber yield and tuber traits of haploid-wild species F1 hybrids", POTATO RESEARCH, ASSOCIATION FOR POTATO RESEARCH, WAGENINGEN, NL, vol. 29, 1 September 1986 (1986-09-01), pages 289-297, XP008132723, ISSN: 0014-3065, DOI: DOI:10.1007/BF02359958

D3 WATANABE K N ET AL: "Generation of pest resistant, diploid potato germplasm with short-day adaptation from diverse genetic stocks", BREEDING SCIENCE, vol. 46, no. 4, 1996, pages 329-336, XP002577156,

D4 WANG D ET AL: "Improving main agronomic characters of dihaploids with 2n gametes by means of sexual hybridisation and anther culture of potato (Solanum tuberosum)", NEW ZEALAND JOURNAL OF CROP AND HORTICULTURAL SCIENCE, vol. 28, no. 1, March 2000 (2000-03), pages 1-8, XP002626196, ISSN: 0114-0671

D5 PHUMICHAI CHALERMPOL ET AL: "Toward the development of highly homozygous diploid potato lines using the self-compatibility controlling Sli gene", GENOME, vol. 48, no. 6, December 2005 (2005-12), pages 977-984, XP002577501, ISSN: 0831-2796

2 The present application appears to meet the criteria of Article 33(1) PCT, because the subject matter of **claims 1 to 7, 14, 15, 21 to 28 and 31 to 33** is new within the meaning of Article 33(2) PCT. While the prior art describes essentially homozygous, diploid, fertile potato plants with tuber yields of at least 200 g per plant (cf. D1, table 8; D2, page 290, "Results", figure 1; D3, table 8; D4, table 3), the cited documents do not test or characterise the self-compatibility of the described plants and therefore the self-compatible, diploid potato plants of claim 1 do not seem to be anticipated by the prior art.

3 D3, which could be regarded as the most relevant prior art for the subject matter of **claim 1**, describes essentially homozygous, diploid, fertile potato plants with tuber yields of at least 200 g per plant, from which the subject matter of claim 1 differs in that it requires the plants to be self-compatible.

The Applicant's attention is drawn to the fact that the claims do not require that the yield is obtained from potato plants grown from seedlings; hence, the plants described in the prior art meet the requirement of an average tuber yield of 200 grams per plant recited in claim 1. The plants of the prior art are likewise considered to be "essentially homozygous", which is defined as being at least 25% homozygous, when testing 100 loci (cf. description, page 21), i. e. the plants need only be homozygous in 25 loci.

The Applicant has contested that the plants described in D3 would meet the requirements of being "essentially homozygous" within the meaning of the description, since this required, according to the Applicant, the testing of 100 loci chosen *randomly* over the whole genome. However, the description, page 21, defines the term "essentially homozygous" as "a level of homozygosity of at least 25% ... homozygosity when testing 100, preferably 1000, more preferably at least 10.000 loci". The description does not require that these loci are chosen randomly. Therefore, the Applicant's argument concerning the homozygosity cannot convince.

Hence, in view of D3, the problem to be solved is the provision of further diploid, essentially homozygous, fertile potato plants with tuber yields of at least 200 g per plant.

The solution proposed by the subject matter of claim 1 consists of plants that are self-compatible.

The Applicant submitted that the problem was "to provide a diploid potato plant that is self-compatible and essentially homozygous and has a high tuber yield also from seedling plants". As already mentioned, the claims do not refer to yield from seedling plants. Since the plants of D3 have a yield of more than 200 g per plant (i. e. up to 600 g per plant; cf. D3, table 8), the yield recited in claim 1 is not a feature differing from the plants of the closest prior art. The same applies to the homozygosity, which is defined by the application as being homozygous in 25 loci (*supra*). Therefore, the only difference to the plants of D3 is their self-compatibility.

The advantages of self-compatible potato plants were *inter alia* known from D5 (cf. D5, page 978, left column), the teaching of which the skilled person would have been aware of, since it is a document from the same technical field. Starting from D3 and having regard of the teaching of D5, the skilled person would have considered the provision of self-compatible potato lines (cf. D5, the whole document). While D5 does not disclose the yield of the self-compatible potato plants, it appears to be self-evident that a breeder is interested in increasing the yield of any given crop. Therefore, the solution proposed by the present application cannot be considered to be inventive.

The same objections apply *mutatis mutandis* to the subject matter of **claims 2 to 7, 14, 15, 22 to 28 and 31 to 33.**

As far as the homozygosity is concerned, it is of course evident that once self-compatible lines are obtained (by following the teaching of D5), an increase in their homozygosity is a mere routine and readily achievable (cf. D5, table 2).

The Applicant has furthermore argued that there was a prejudice against breeding homozygous breeding lines, relying on a document from 1987 and an internet disclosure, both of which were however not submitted. Consequently, it is impossible to assess their teaching. However, a prejudice in the art is usually defined as an opinion widely or universally held by experts in the field, normally demonstrated by reference to reviews or encyclopedias. It appears that the existence of such a prejudice has not been sufficiently proven in the present case.

4 Claim 21 relates to deposited diploid, fertile, self-compatible potato plants with different degrees of homozygosity. Their yield is characterised as intermediate/high, although exact data are not disclosed in the application.

Document D5, which is considered to represent the most relevant state of the art for the subject matter of claim 21, discloses (cf. D5, figure 1, table 1) diploid, fertile, self-compatible, homozygous potato plants (inbred series B), from which the subject matter of claim 21 differs in that provides apparently genetically different diploid potato plants.

The problem to be solved by the present invention may therefore be regarded as the provision of further diploid potato plants.

The solution proposed in **claim 21** of the present application cannot be considered as involving an inventive step (Article 33(3) PCT), since the skilled person obviously would have considered the provision of further diploid homozygous self-compatible potato lines based e. g. on the teaching of document D5 (cf. D5, figure 1, table 1). It is emphasized that the yield of the deposited lines is not quantitated and that some lines are only 25% homozygous. Hence, the Applicant's arguments concerning the yield and the homozygosity, submitted in reply to the Written Opinion of the International Searching Authority, do not appear to apply to (some of) the deposited lines. The deposited lines do not appear to be associated with any unexpected or surprising technical effect.

5 According to the PCT Guidelines, A5.26[1], claims for products defined in terms of a process of manufacture (cf. **claims 14, 15, 22 to 28, 31, 33**) are to be construed as claims to the product as such. A product is not rendered novel merely by the fact that it is produced by means of a new process.

6 The attention of the Applicant is drawn to the fact that the application may not be amended in such a way that it contains subject matter which extends beyond the content of the application as filed (Article 34(2)(b) PCT).

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 May 2011 (05.05.2011)

PCT

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(71) Applicant *(for all designated States except US)*: **AGVENTURE B.V.** [NL/NL]; Burg. Arriensweg 32, NL-7431 BM Diepenveen (NL).

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(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.

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(54) Title: HYBRID SEED POTATO BREEDING

(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 preparing such plants and to hybrid seeds obtained by crossing such homozygous potato lines and to potato plants grown from said see.

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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

5

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, 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.

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-e 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

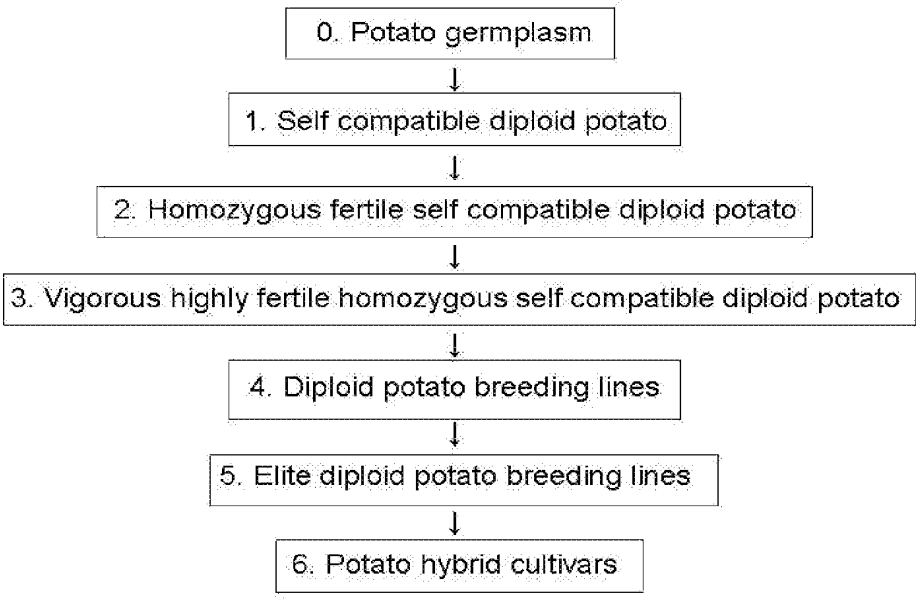
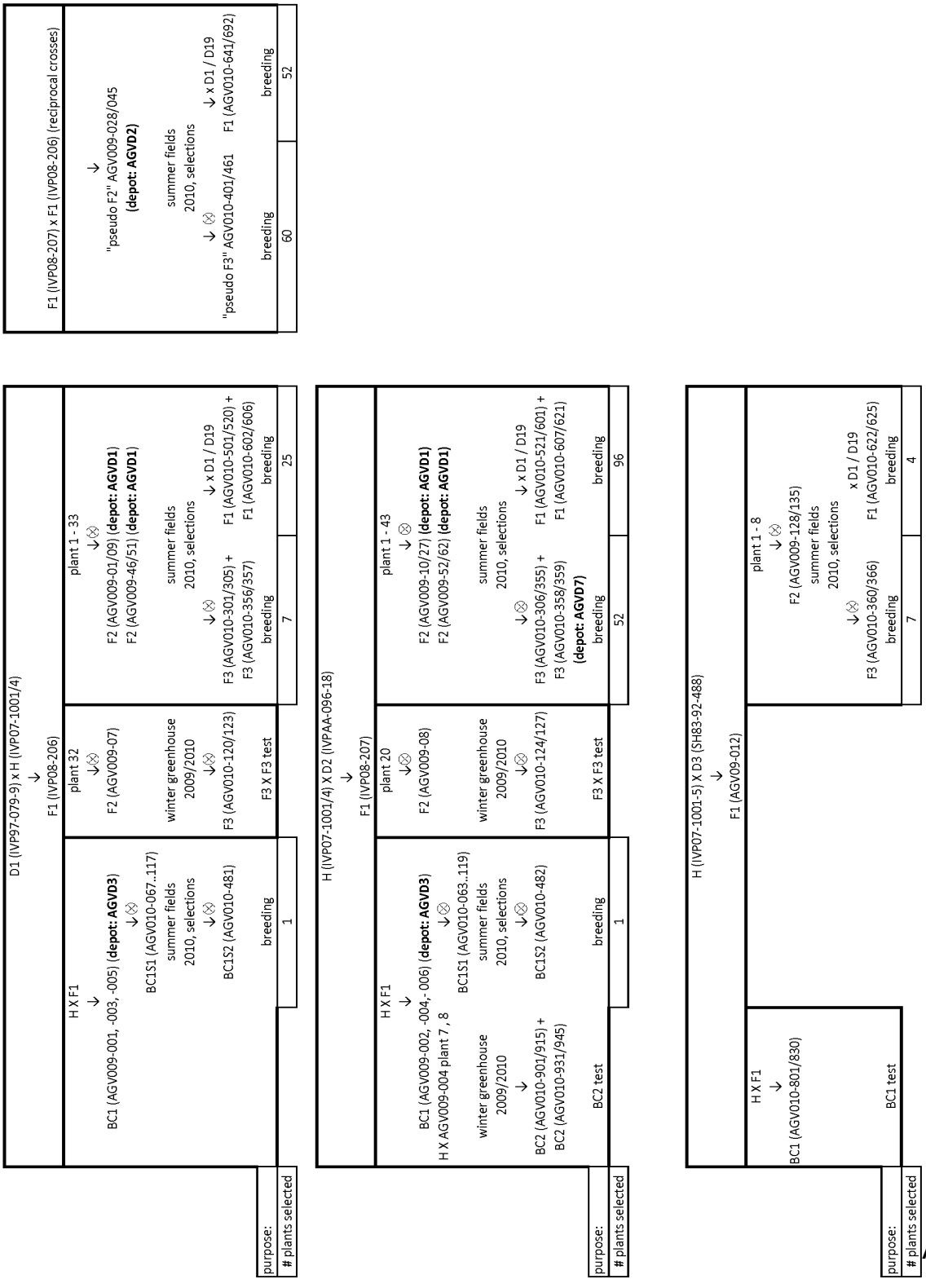

0. Potato germplasm
1. Self compatible diploid potato
2. Homozygous fertile self compatible diploid potato
3. Vigorous highly fertile homozygous self compatible diploid potato
4. Diploid potato breeding lines
5. Elite diploid potato breeding lines
6. Potato hybrid cultivars

Figure 2



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Figure 3

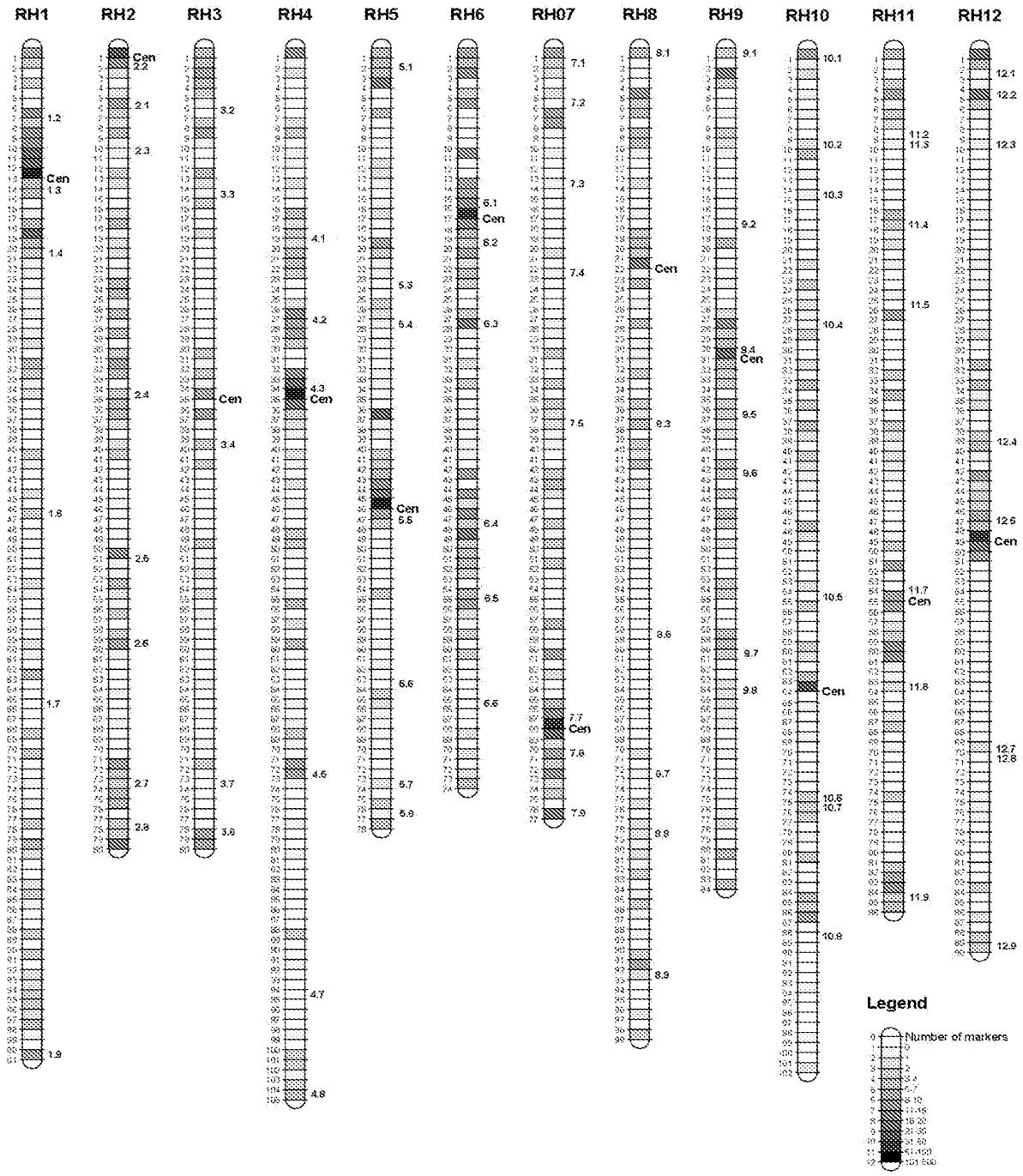
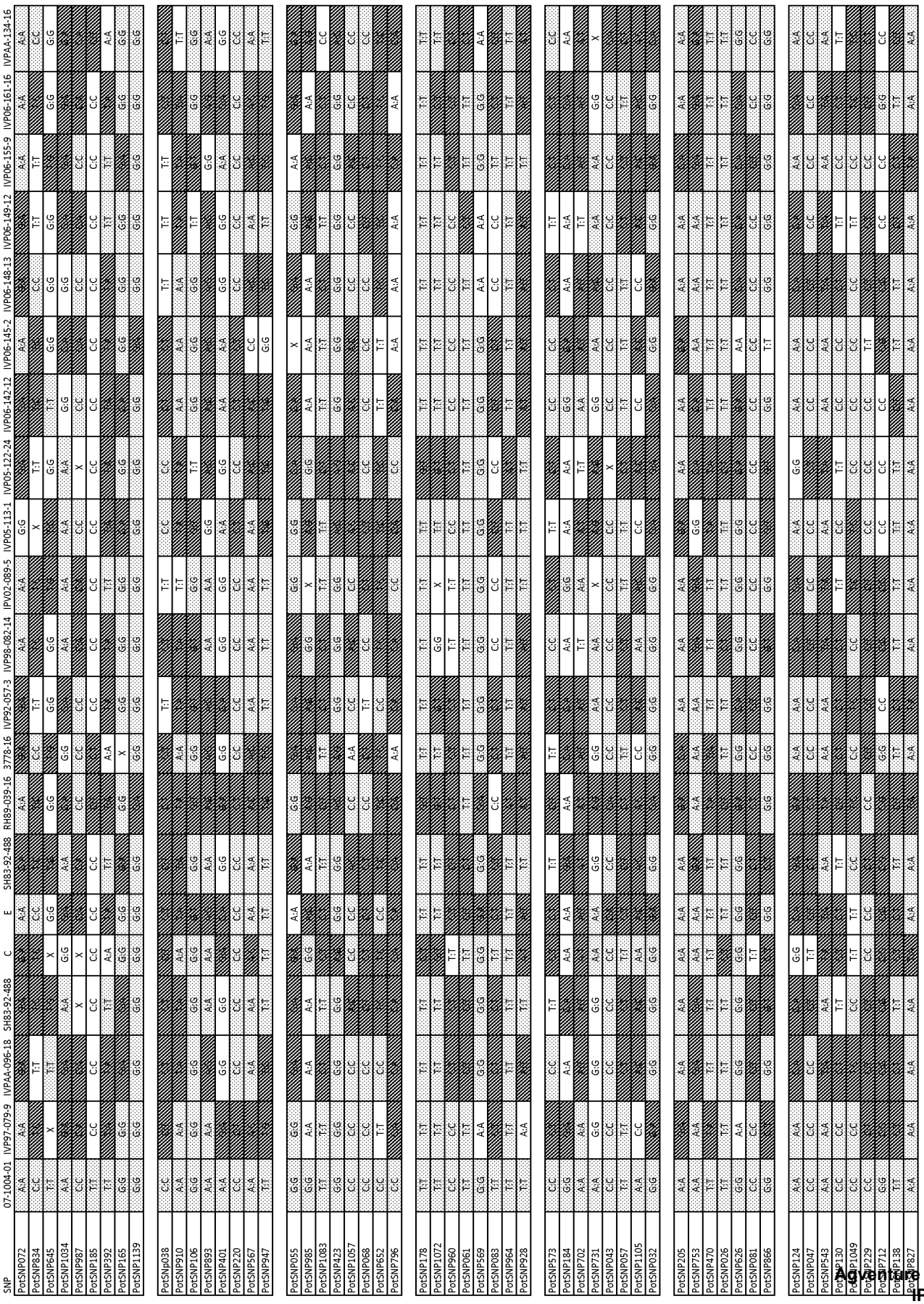
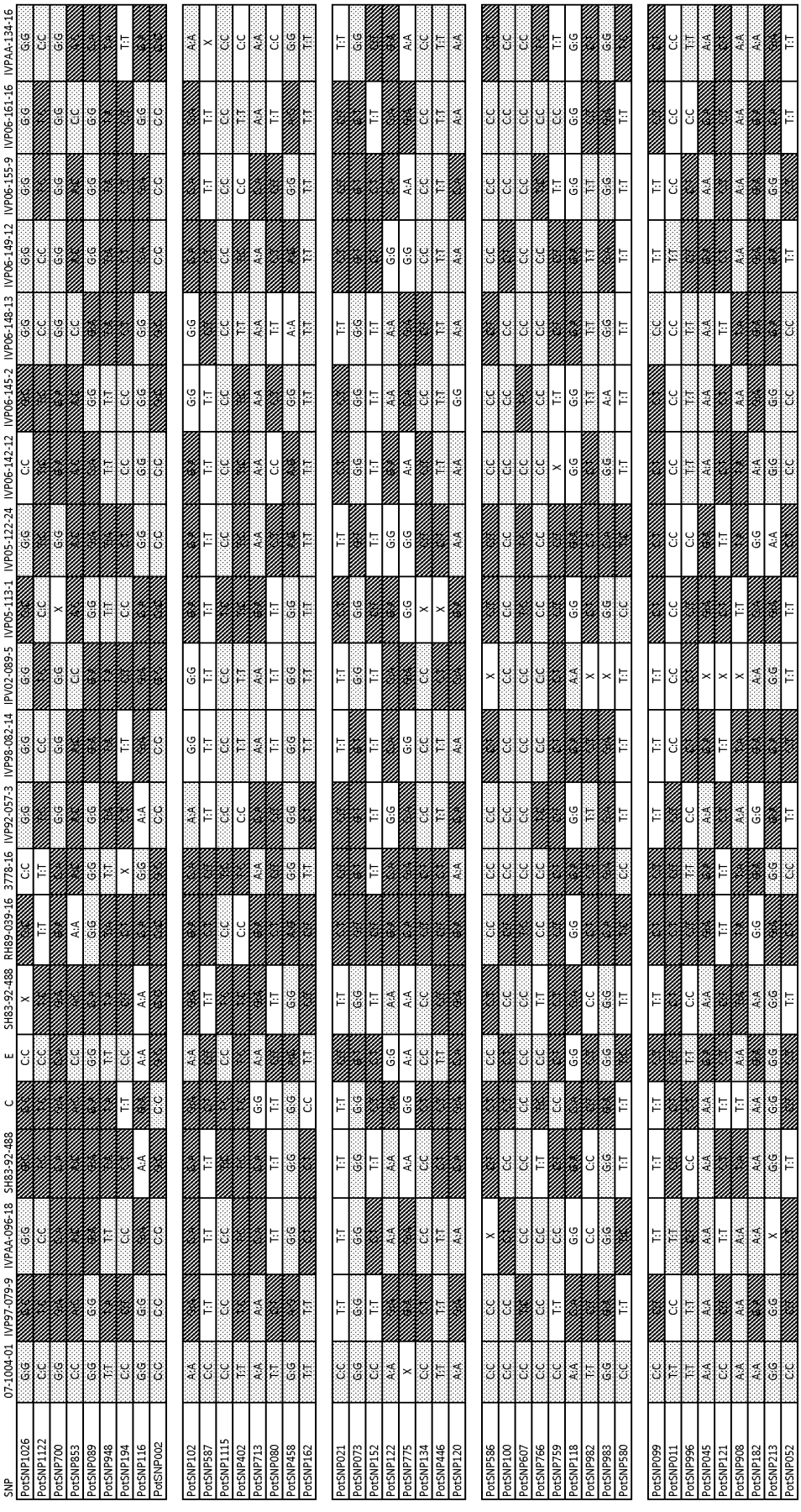

RH1
RH2
RH3
RH4
RH5
RH6
RH07
RH8
RH9
RH10
RH11
RH12
Cen
Legend
Number of markers

Figure 4



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Figure 4 continued



SNP	07-1004-01	IVP97-079-9	IVPAA-096-18	SH83-92-488	C	E	SH83-92-488	RH89-039-16	3778-16	IVP92-057-3	IVP98-082-14	IPVO2-089-5	IVP05-113-1	IVP05-122-24	IVP06-142-12	IVP06-145-2	IVP06-148-13	IVP06-149-12	IVP06-155-9	IVP06-161-16	IVPAA-134-16
PotSNP1026	G:G	A:A	G:G	A:A	C:C	A:A	C:C	X	A:A	G:G	G:G	G:G	A:A	G:G	C:C	G:G	G:G	G:G	G:G	G:G	G:G
PotSNP1122	C:C	X	C:C	X	C:C	X	T:T	T:T	C:C	G:G	C:C	X	C:C	X	X	C:C	C:C	X	X	X	C:C
PotSNP700	G:G	X	X	X	X	X	X	X	X	G:G	G:G	G:G	X	G:G	X	G:G	G:G	G:G	G:G	X	G:G
PotSNP853	C:C	X	X	X	X	C:C	A:A	A:A	X	X	X	C:C	G:G	X	X	C:C	X	X	C:C	C:C	X
PotSNP089	G:G	G:G	X	X	G:G	G:G	G:G	G:G	X	G:G	X	X	G:G	X	X	G:G	X	G:G	G:G	G:G	X
PotSNP948	T:T	X	T:T	X	T:T	X	G:G	X	T:T	X	X	X	X	T:T	X	X	X	X	X	X	T:T
PotSNP194	C:C	X	C:C	X	C:C	T:T	X	T:T	X	X	T:T	X	X	C:C	X	C:C	G:G	X	X	X	X
PotSNP116	G:G	G:G	X	A:A	A:A	X	G:G	X	A:A	X	X	X	G:G	G:G	G:G	G:G	G:G	X	G:G	G:G	A:A
PotSNP002	C:C	C:C	C:C	X	X	X	X	X	C:C	X	X	X	C:C	C:C	C:C	X	X	C:C	C:C	C:C	G:G
PotSNP102	A:A	X	X	X	X	A:A	X	X	A:A	G:G	G:G	G:G	X	G:G	X	G:G	G:G	X	X	X	A:A
PotSNP587	C:C	T:T	T:T	T:T	X	X	T:T	X	T:T	T:T	T:T	T:T	T:T	T:T	T:T	T:T	X	T:T	T:T	T:T	X
PotSNP1115	C:C	C:C	C:C	X	X	C:C	X	X	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
PotSNP402	T:T	X	X	X	X	C:C	X	X	T:T	T:T	T:T	T:T	X	X	X	X	T:T	X	X	T:T	C:C
PotSNP713	A:A	A:A	X	X	G:G	A:A	A:A	X	A:A	A:A	A:A	A:A	X	A:A	A:A	A:A	A:A	X	X	A:A	A:A
PotSNP080	T:T	X	T:T	T:T	T:T	A:A	X	X	T:T	T:T	T:T	T:T	T:T	C:C	X	T:T	T:T	T:T	T:T	T:T	C:C
PotSNP458	G:G	X	G:G	G:G	G:G	X	G:G	X	G:G	G:G	G:G	G:G	G:G	X	G:G	G:G	A:A	X	G:G	X	G:G
PotSNP162	T:T	X	X	X	C:C	T:T	X	X	T:T	T:T	T:T	T:T	T:T	X	X	T:T	T:T	X	X	X	T:T
PotSNP021	C:C	T:T	T:T	T:T	T:T	X	T:T	X	T:T	T:T	T:T	T:T	X	T:T	X	X	T:T	X	X	X	T:T
PotSNP073	G:G	G:G	G:G	G:G	G:G	X	G:G	X	G:G	G:G	G:G	G:G	G:G	G:G	G:G	X	G:G	X	X	X	G:G
PotSNP152	C:C	T:T	T:T	T:T	T:T	X	T:T	X	T:T	T:T	T:T	T:T	X	T:T	T:T	T:T	T:T	X	X	X	X
PotSNP122	A:A	X	A:A	A:A	A:A	X	A:A	X	X	G:G	X	X	X	G:G	A:A	A:A	A:A	G:G	X	X	X
PotSNP775	X	X	X	A:A	G:G	A:A	A:A	X	X	X	G:G	G:G	G:G	G:G	A:A	X	X	G:G	A:A	X	A:A
PotSNP134	C:C	C:C	C:C	C:C	C:C	C:C	C:C	X	C:C	C:C	C:C	C:C	X	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
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PotSNP120	A:A	X	A:A	X	X	X	X	X	A:A	X	A:A	X	X	A:A	A:A	A:A	A:A	X	X	X	A:A
PotSNP586	C:C	C:C	X	X	X	C:C	X	C:C	C:C	C:C	X	X	X	C:C	C:C	C:C	C:C	C:C	C:C	C:C	X
PotSNP100	C:C	C:C	X	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
PotSNP607	C:C	X	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C
PotSNP766	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	C:C	X	C:C	C:C	C:C	C:C	C:C	X
PotSNP759	C:C	T:T	C:C	X	C:C	T:T	X	X	C:C	X	C:C	C:C	C:C	C:C	T:T	C:C	C:C	T:T	C:C	T:T	T:T
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PotSNP982	T:T	X	C:C	C:C	C:C	X	C:C	X	T:T	X	X	X	G:G	T:T	T:T	T:T	T:T	T:T	T:T	X	X
PotSNP983	G:G	X	G:G	G:G	G:G	G:G	G:G	X	G:G	X	X	X	G:G	G:G	A:A	A:A	G:G	X	G:G	X	G:G
PotSNP580	C:C	T:T	X	T:T	T:T	X	T:T	X	C:C	T:T	T:T	T:T	C:C	T:T	T:T	T:T	T:T	T:T	T:T	T:T	X
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PotSNP045	A:A	A:A	A:A	A:A	A:A	X	A:A	X	X	A:A	X	X	X	X	A:A	A:A	A:A	X	X	A:A	A:A
PotSNP121	C:C	X	T:T	X	T:T	X	T:T	X	T:T	X	T:T	X	X	T:T	X	X	T:T	X	X	X	X
PotSNP908	A:A	A:A	X	T:T	A:A	X	X	X	X	A:A	X	X	T:T	X	X	A:A	X	A:A	A:A	A:A	A:A
PotSNP182	A:A	X	A:A	A:A	X	X	A:A	G:G	X	G:G	A:A	A:A	X	G:G	A:A	G:G	X	G:G	G:G	G:G	A:A
PotSNP213	G:G	G:G	X	G:G	G:G	G:G	G:G	X	G:G	X	G:G	G:G	A:A	X	G:G	G:G	X	X	G:G	X	X
PotSNP052	C:C	X	X	T:T	X	X	T:T	X	C:C	T:T	T:T	T:T	T:T	X	C:C	C:C	X	T:T	X	T:T	T:T

Figure 5 A

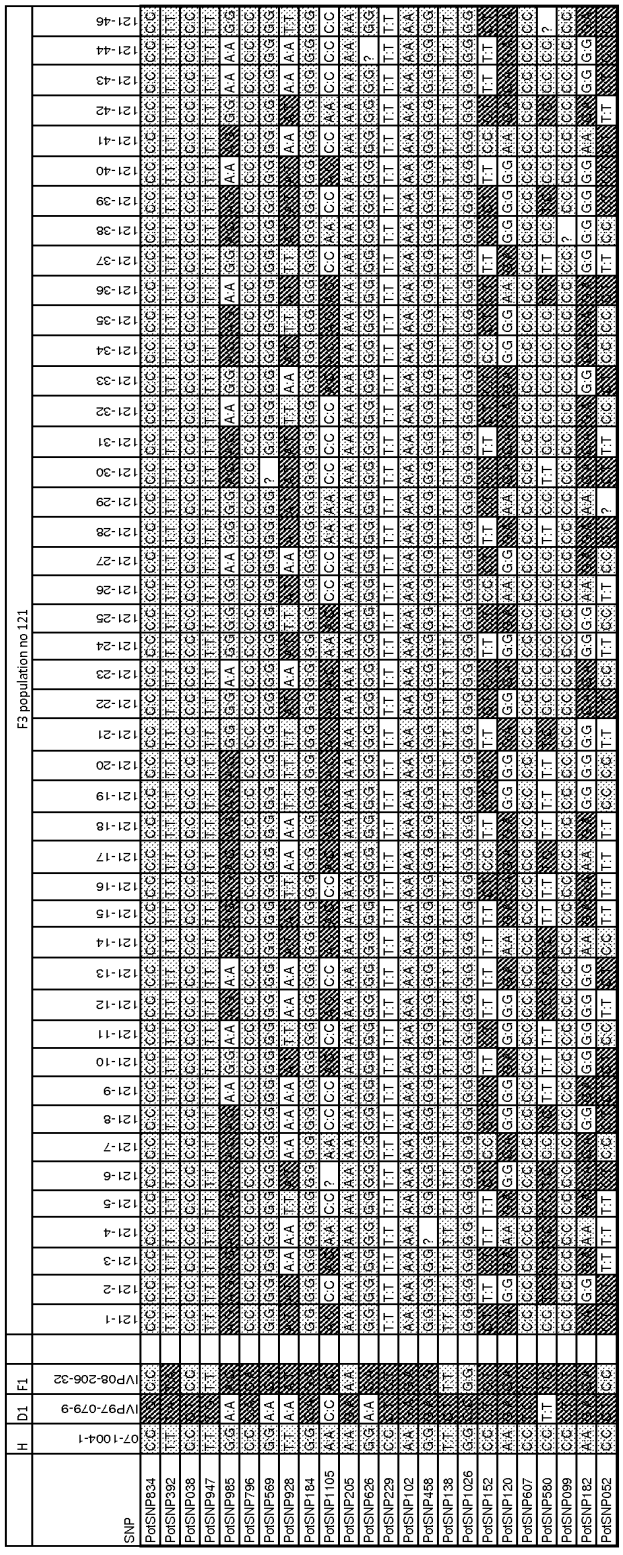

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Figure 5 B

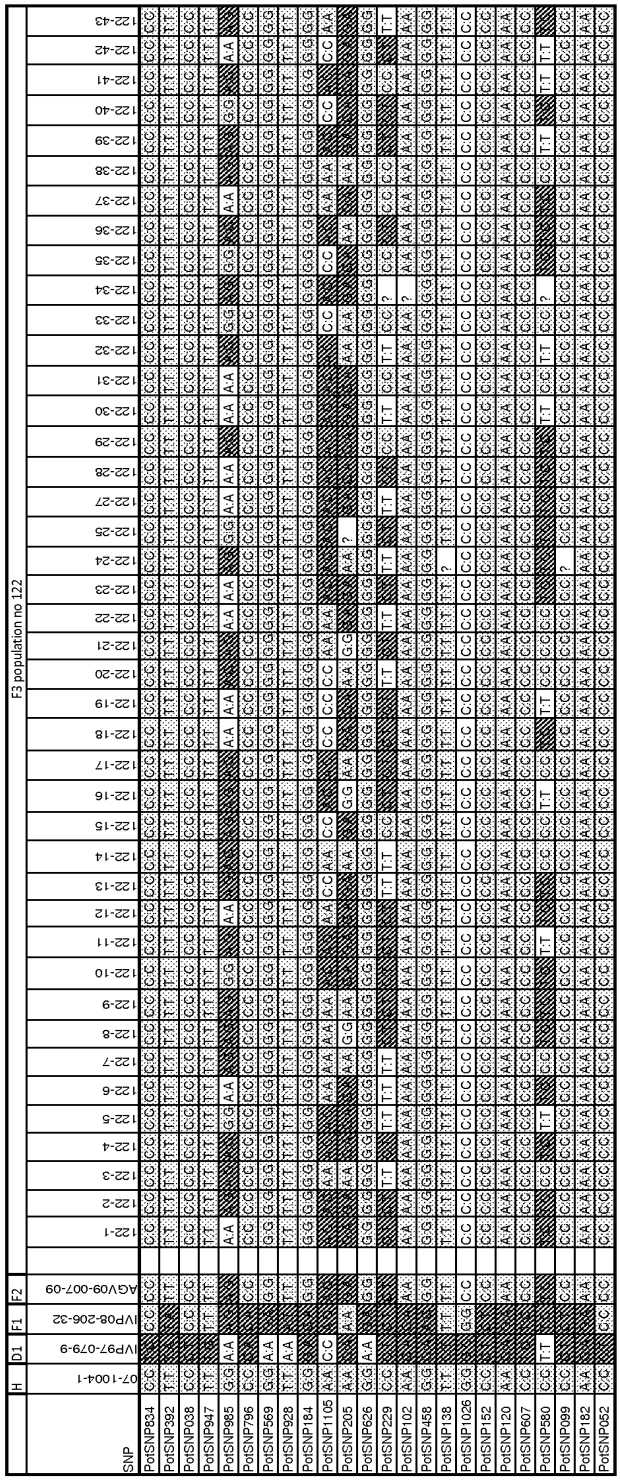

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Figure 5 C

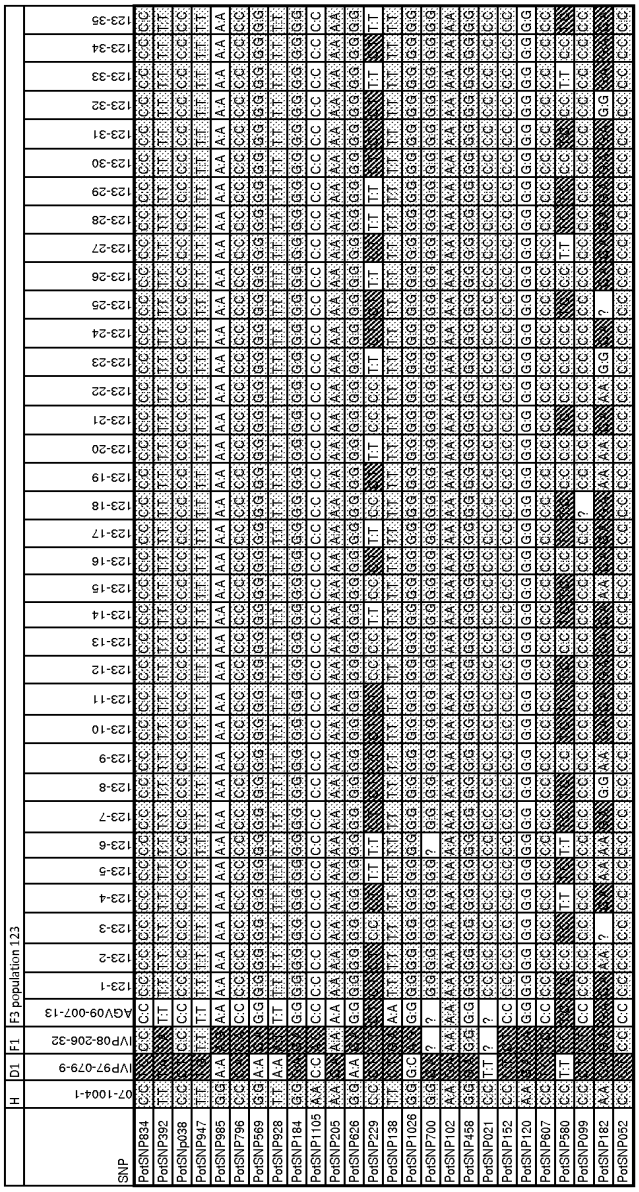

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Figure 5 D

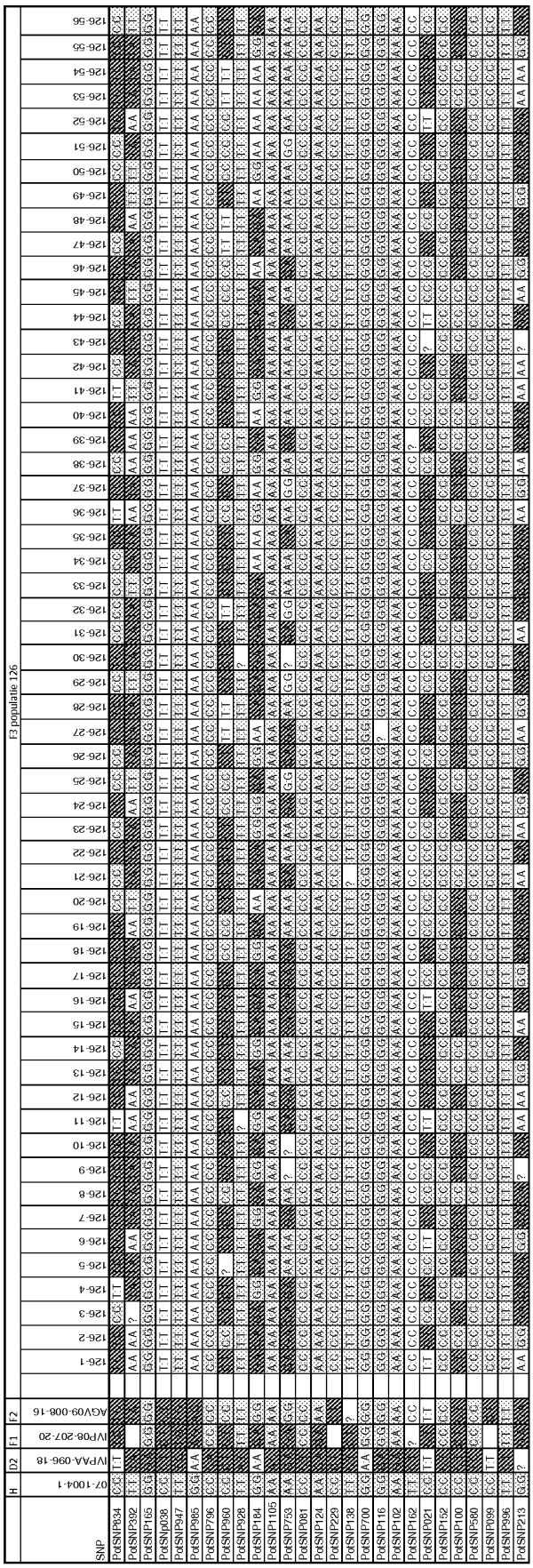

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Figure 5 E

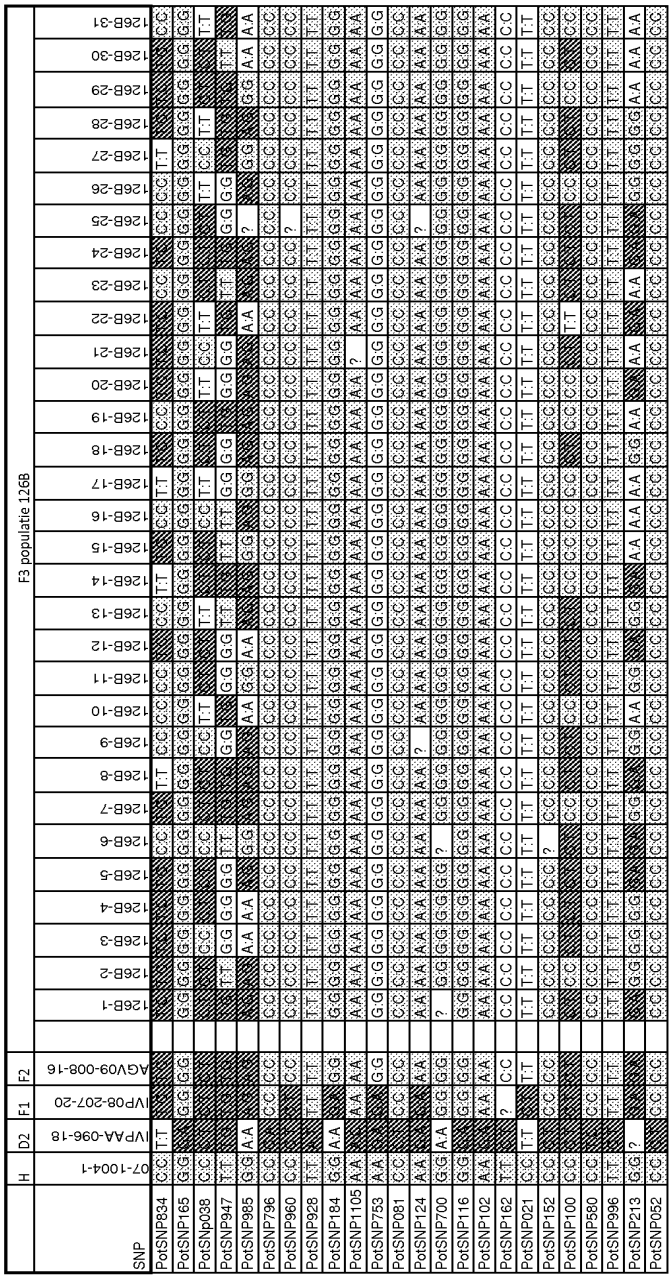
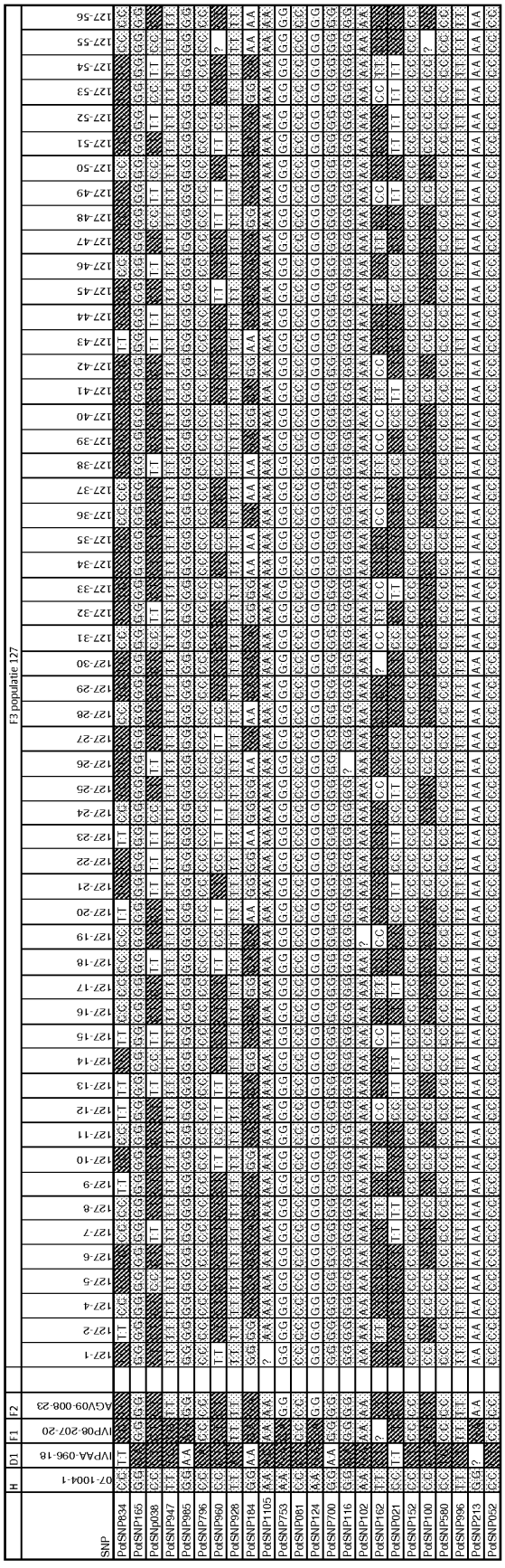

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Figure 5 F


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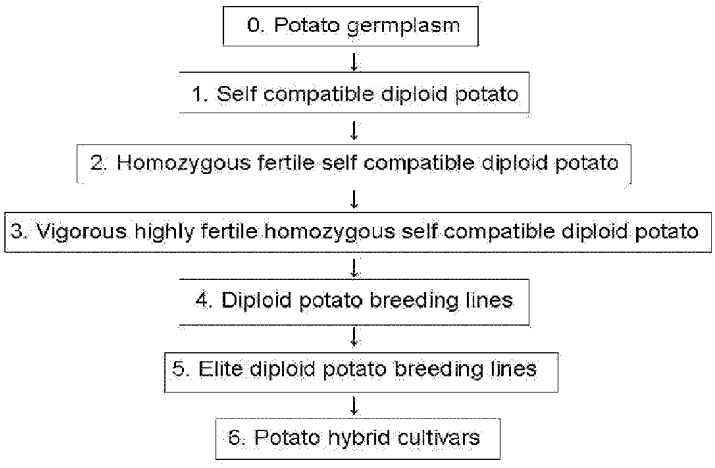
Published:

— *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

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U.S. APPLICATION NUMBER NO.	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
13/504,156	Willem Hendrik Lindhout	05032-00255

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OC000000054198179

Date Mailed: 05/10/2012

NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371 IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

The following items have been submitted by the applicant or the IB to the United States Patent and Trademark Office as a Designated Office (37 CFR 1.494):

- Indication of Small Entity Status
- Priority Document
- Copy of the International Application filed on 04/26/2012
- Copy of the International Search Report filed on 04/26/2012
- Copy of IPE Report filed on 04/26/2012
- Preliminary Amendments filed on 04/26/2012
- U.S. Basic National Fees filed on 04/26/2012
- Assignee Statement for PGPUB filed on 04/26/2012
- Priority Documents filed on 04/26/2012

The applicant needs to satisfy supplemental fees problems indicated below.

The following items **MUST** be furnished within the period set forth below in order to complete the requirements for acceptance under 35 U.S.C. 371:

- Oath or declaration of the inventors, in compliance with 37 CFR 1.497(a) and (b), identifying the application by the International application number and international filing date.
- To avoid abandonment, a surcharge (for late submission of filing fee, search fee, examination fee or oath or declaration) as set forth in 37 CFR 1.492(h) of $65 for a small entity in compliance with 37 CFR 1.27, must be submitted with the missing items identified in this letter.

SUMMARY OF FEES DUE:

Total additional fees required for this application is $**65** for a Small Entity:

- $**65** Surcharge.
- This application clearly fails to comply with the requirements of 37 CFR. 1.821-1.825. Applicant's attention is directed to the final rulemaking notice published at 55 FR 18230 (May 1, 1990), and 1114 OG 29 (May 15, 1990). If the effective filing date is on or after July 1, 1998, see the final rulemaking notice published at 63 FR 29620 (June 1, 1998) and 1211 OG 82 (June 23, 1998). If the effective filing date is on or after September 8, 2000, see the final rulemaking notice published in the Federal Register at 65 FR 54604 (September 8, 2000) and 1238 OG 145 (September 19, 2000). Applicant must provide an initial computer readable form (CRF) copy of the "Sequence Listing", an initial paper or compact disc copy of the "Sequence Listing", **as well as an amendment specifically directing its entry into the application**. Applicant must also provide a

Agventure B.V. Ex. 2009
IPR2025-00136
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statement that the content of the sequence listing information recorded in computer readable form is identical to the written (on paper or compact disc) sequence listing and, where applicable, includes no new matter, as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b), or 1.825(d). If applicant desires the sequence listing in the instant application to be identical with that of another application on file in the U.S. Patent and Trademark Office, such request in accordance with 37 CFR 1.821(e) may be submitted in lieu of a new CRF.

- A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 CFR 1.821(e). If the effective filing date is on or after September 8, 2000, see the final rulemaking notice published in the Federal Register at 65 FR 54604 (September 8, 2000) and 1238 OG 145 (September 19, 2000). Applicant must provide an initial computer readable form (CRF) copy of the "Sequence Listing" and a statement that the content of the sequence listing information recorded in computer readable form is identical to the written (on paper or compact disc) sequence listing and, where applicable, includes no new matter, as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b), or 1.825(d). If applicant desires the sequence listing in the instant application to be identical with that of another application on file in the U.S. Patent and Trademark Office, such request in accordance with 37 CFR 1.821(e) may be submitted in lieu of a new CRF.

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.

For questions regarding compliance to 37 CFR 1.821-1.825 requirements, please contact:

- **For Rules Interpretation, call (571) 272-0623**
- **For Patentin Software Program Help, call Patent EBC at 1-866-217-9197 or directly at 703-305-3028 / 703-308-6845 between the hours of 6 a.m. and 12 midnight, Monday through Friday, EST.**
- **Send e-mail correspondence for Patentin Software Program Help @ ebc@uspto.gov**

ALL OF THE ITEMS SET FORTH ABOVE MUST BE SUBMITTED WITHIN TWO (2) MONTHS FROM THE DATE OF THIS NOTICE OR BY 32 MONTHS FROM THE PRIORITY DATE FOR THE APPLICATION, WHICHEVER IS LATER. FAILURE TO PROPERLY RESPOND WILL RESULT IN ABANDONMENT.

The time period set above may be extended by filing a petition and fee for extension of time under the provisions of 37 CFR 1.136(a).

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

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JOHN L ANDERSON

Telephone: (571) 272-0385

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 13/504,156

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	190		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	245		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	125		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	34 minus 20 =	* 14	x 30 =	420	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	7 minus 3 =	* 4	x 125 =	500			
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	1480		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.
** 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.

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

Substitute for Form PTO-1360
(For use with Form PTO/SB/06)

Application Number	Filing Date
13504156	

Applicant(s) Willem Lindhout

* May be used for additional claims or amendments

CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT	
	Indep	Depend	Indep	Depend	Indep	Depend
1	1		1			
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	13504156
Filing Date	2012-04-26
First Named Inventor	Willem Hendrick Lindhout
Art Unit	TBA
Examiner Name	TBA
Attorney Docket Number	05032-00255

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	1	0913081	EP	A1	1999-05-06	President of Kobe University		☐

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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	13504156
	Filing Date	2012-04-26
	First Named Inventor	Willem Hendrick Lindhout
	Art Unit	TBA
	Examiner Name	TBA
	Attorney Docket Number	05032-00255

	1	International Search Report relating to corresponding PCT/NL2010/050716, issued June 8, 2011.	☐
	2	International Preliminary Report on Patentability relating to corresponding PCT/NL2010/050716, issued April 26, 2012.	☐
	3	BANI-AAMEUR, et al., "Enhancement of Diploid Solanum Chacoense Bitt. Using Adapted Clones of Solanum Phureja Juz. & Buk.," Euphytica, 68: 169-179, 1993.	☐
	4	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).	☐
	5	DeMAINE, M. J., "The Effects of Inbreeding on the Parental Values of Potato Dihaploids," Ann. Appl. Biol. (1995) 127: 151-156.	☐
	6	DINU, et al., "Novel Inter-Series Hybrids in Solanum, Section Petota," Theor Appl Genet (2005) 110: 403-415.	☐
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	8	HERMSEN, et al., "Inheritance of Genetic Markers From Two Potato Dihaploids and Their Respective Parent Cultivars," Euphytica, 27 (1978) 681-688.	☐
	9	HERMUNDSTAD, et al., "Tuber Yield and Tuber Traits of Haploid-Wild Species F1 Hybrids," Potato Research 29 (1986) 289-297.	☐
	10	HOSAKA, et al., "Genetics of Self-Compatibility Wild Diploid Potato Species Solanum Chacoense. 1. Detection of an S Locus Inhibitor (Sli) Gene," Euphytica, 99: 191-197, 1998.	☐
	11	JANSKY, S., "Overcoming Hybridization Barriers in Potato," Plant Breeding, 125, 1-12 (2006).	☐

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	Application Number	13504156
	Filing Date	2012-04-26
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	Art Unit	TBA
	Examiner Name	TBA
	Attorney Docket Number	05032-00255

	12	MARIS, B., "Comparison of Diploid and Tetraploid Potato Families Derived from Solanum Phureja x Dihaploid S. Tuberosum Hybrids and Their Vegetatively Doubled Counterparts," Euphytica, 46: 15-33, 1990.	☐
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	16	VISKER, et al., "Correlation Between Late Blight Resistance and Foliage Maturity Type in Potato," Euphytica, 137: 311-323, 2004.	☐
	17	WANG, et al., "Improving Main Agronomic Characters of Dihaploids with 2n Gametes by Means of Sexual Hybridisation and Another Culture of Potato (Solanum Tuberosum)," New Zealand Journal of Crop and Horticultural Science, 2000, Vol. 28: 1-8.	☐
	18	WATANABE, et al., "Generation of Pest Resistant. Diploid Potato Germplasm with Short-Day Adaptation from Diverse Genetic Stocks," Breeding Science, 46: 329-336 (1996).	☐

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EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

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	Application Number	13504156
	Filing Date	2012-04-26
	First Named Inventor	Willem Hendrick Lindhout
	Art Unit	TBA
	Examiner Name	TBA
	Attorney Docket Number	05032-00255

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ 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	/John P. Iwanicki/	Date (YYYY-MM-DD)	2012-05-22
Name/Print	John P. Iwanicki	Registration Number	34628

This collection of information is required by 37 CFR 1.97 and 1.98. 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 1 hour 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.**

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(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 913 081 A1

(12) EUROPEAN PATENT APPLICATION

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06.05.1999 Bulletin 1999/18

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(74) Representative: **HOFFMANN - EITLE**
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(54) Method of producing a mini-potato

(57) Solanum stenotomum, Solanum phureja and Solanum goniocalyx, which are diploid potatoes native to the Andes, are crossed by open pollination, thereby forming a hybrid population rich in genetic variation. The hybrid population is raised while screening the hybrid population on the basis of selection criteria including a yield thereof, thereby reconstituting the hybrid population. The reconstituted hybrid population is raised for seedlings under short-day light conditions while limiting a rhizosphere thereof and controlling the growth at a slightly lower temperature during a potato-tuber growing period. A mini-potato edible whole (including skin) in one bite can be thus obtained.

EP 0 913 081 A1

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Description

[0001] The present invention relates to a novel potato species capable of providing a mini-potato, which is a mini-tomato-size potato and edible whole (including rind on skin) in one bite, and a method of producing the mini-potato by raising the novel potato species.

[0002] Hitherto, growing large farm products has been a basic strategy in the agricultural field. In contrast, small farm products were evaluated and treated as low grade products. However, since eating habits have changed in recent days, the small farm products are rather valued in some cases. A mini-tomato edible in one bite is a representative example of successful small farm products. However, not so many successful small farm products other than the mini-tomato are known at present.

[0003] With respect to potatoes, a mini-tomato-size potato which is edible whole (including rind on skin) in one bite, has not yet been produced. Potatoes are therefore usually peeled, cut into an appropriate size, and then cooked. In the case of a baked potato, the potato is cooked (baked) and then cut into bite-size pieces at the table. Although immature potato tubers (about 30g weight) called "small potato" can be produced when a potato plant is grown in a general cultivation method, the size of the small potato is still too large to be used whole in snack foods.

[0004] Then, if it is possible to produce such a mini-potato edible whole (including skin) in one bite, similarly to the mini-tomato, the mini-potato will be valuable as a raw food material for snack confectioneries and as snacks taken with beer.

[0005] An object of the present invention is to provide a method of producing a mini-tomato-size potato called a mini-potato which is edible whole (including skin) in one bite.

[0006] The object described above is achieved by the method of producing a bite-size mini-potato comprising the steps of:

crossing Solanum stenotomum, Solanum phureja and Solanum goniocalyx, which are diploid potatoes native to the Andes, by open pollination, thereby forming a hybrid population rich in genetic variation;
effecting breeding cultivation of the hybrid population and screening the hybrid population on the basis of selection criteria including a yield thereof, thereby reconstituting the hybrid population; and
effecting seedling cultivation of the reconstituted hybrid population to obtain seeds, the seedling cultivation being effected under short-day light conditions while limiting a rhizosphere and controlling the growth at a slightly low temperature during a potato-tuber growth period.

[0007] In the present invention, diploid species, Solanum stenotomum, Solanum phureja, and Solanum gonicalyx are used as starting species. This is because the potatoes produced from these diploid species are smaller than those from an ordinary species (tetraploid) and therefore suitable for forming a new species for the mini-potato. A first population is formed by performing open pollination among the three species. This is because it is rather better to screen a potato species starting from a population rich in genetic variation in order to make the best of various shapes and colors of the first population in the resultant food material. When the novel potato species of the present invention is formed, the population is reconstituted by repeating the crossing (bulk-pollen mixed pollination) and screening. This is made to eliminate unpreferable traits (particularly an acrid taste).

[0008] In the mini-potato producing method of the present invention, a rhizosphere is limited. This is made to obtain small potato-tubers easily. To limit the rhizosphere, a pot and a culture tray is used.

[0009] In the method of the present invention, the seedling cultivation of the hybrid population is effected under short-day light conditions. This is because the potato-tubers of the diploid species is are initiated under the short-day conditions. However, if the diploid species is grown under long-day conditions, an aboveground portion of the potato plant grows well but the potato-tubers underground do not grow. The short-day conditions used herein correspond to the conditions under a natural day-length of autumn to winter.

[0010] Growth of the potato plant is controlled at a slightly lower temperature ranging from 20°C to 5°C, and more preferably at about 10°C during the potato-tuber growing period. In this case, it is necessary to use an unheated glass house or a vinyl house in order to prevent frost damage.

[0011] Now, the present invention will be explained in detail with reference to the following example.

EXAMPLE

[0012] A novel potato species of the present invention was produced and then the mini-potato was obtained by growing the seeds of the novel potato species, as follows.

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(1) The Formation of Population

[0013] Diploid species (1430 individuals, in total) from the following potato species native to the Andes were planted at random in a cultivated field. Seeds were obtained by open pollination and used as a starting population.

Solanum stenotomum:
45 individuals taken from each of 14 families
Solanum phureja:
25 individuals taken from each of 27 families
Solanum goniocalyx:
25 individuals taken from each of 5 families

(II) Breeding

[0014] Three cycles of selection were carried out to reconstitute the aforementioned population and form a population capable of producing, in as large an amount as possible, a mini-tomato-size mini-potato without an acrid taste while maintaining genetic diversity in shape and color. The selection process will be explained.

〈First Selection 〉

1992

[0015]

September 15: Gibberellin treatment of the seeds
Seeds were immersed in 2000 ppm gibberellin GA 3, overnight.
September 16 : Sowing the seeds October 5 to 10: Transplanting
Individuals (1729) were transplanted in a black poly-pot (10.5 cm diameter) and grown by ordinary manners in an unheated glass house.

1993

[0016]

January 14-18: Harvesting of potatoes
Potatoes were harvested from each of 1591 individuals.
January 28: Selection
The harvested potatoes were checked and selected in accordance with selection criteria* described later. 438 individuals were selected.
March 2: Planting ubers
Unsprouted 183 individuals were planted in a heated greenhouse.
April 1 to 9: Transplanting
126 emerged individuals were transplanted to 6 inches clay pots and placed in an unheated screen house.
May 14 to 21: Pollination
Pollen was collected from 109 blooming individuals to make a pollen mixture. 544 flowers in total were pollinated with the pollen mixture.
June 18-24: Collectiing berries

* The selection carried out on January 28 was made by discarding individuals which produced potatoes satisfying the following conditions.
(1) Individuals whose buds have already extended;
(2) Individuals producing extremely small potatoes alone;
(3) Individuals whose products have deep eyes;
(4) Individuals whose products (potatoes) are not uniform in size;
(5) Individuals whose products (potatoes) have unacceptable shapes;
(6) Individuals whose yield is low compared to others;
(7) Individuals whose products have still clear stolon-end portions.

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390 berries were collected. One month later, seeds were extracted from the harvested berries.

〈Second Selection〉

1993

[0017]

September 8:	Gibberellin treatment of the seeds Seeds obtained in the first selection were immersed in 2000 ppm gibberellin GA 3, overnight.
September 9:	Sowing seeds
September 24-27:	Transplanting Individuals (2881) were transplanted in a black poly-pot (10.5 cm diameter) and grown by ordinary manners in an unheated glass house.

1994

[0018]

January 5-6:	Harvesting of potatoes Potatoes were harvested from each of 2442 individuals.
January 12-13:	Selection The harvested potatoes were checked and selected in accordance with the same selection criteria* as used in the first selection test. 719 individuals were selected.
Late in January:	Taste test One potato was taken from each of the individuals, steamed and tasted. As a result, 365 potatoes presenting no acrid taste were selected.
March 2:	Planting tubers 201 unsprouted individuals were planted in a heated greenhouse.
March 31:	Transplantig 127 emerged individuals were transplanted to 6 inches clay pots and grown by ordinary manners in an unheated screen house.
May 9 to 19:	Pollination Pollen was collected from 121 blooming individuals to make a pollen mixture. 290 flowers in total were pollinated with the pollen mixture.
June 18-24:	Collecting berries 203 berries were collected. One month later, seeds were extracted from the harvested fruits.

〈Third Selection〉

1994

[0019]

September 5:	Gibberellin treatment of the seeds Seeds obtained in the second selection were immersed in 2000 ppm gibberellin GA 3, overnight.
September 6:	Sowing seeds in cell trays
October 12-24:	Transplanting Individuals (1800) were transplanted in a black poly-pot (10.5 cm diameter) and grown by ordinary manners in an heated glass house.

1995

[0020]

January 9-10	Harvesting of potatoes Potatoes were harvested from each of 1772 individuals.
January 25-26:	Selection

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	The harvested potatoes were checked and selected in accordance with the same selection criteria* as used in the first selection. 465 individuals were selected.
February 20:	Taste test One potato was taken from each of 339 unsprouted individuals steamed, and tasted. As a result, 240 potatoes without an acrid taste were selected.
March 7:	Planting tubers 162 unsprouted individuals were transplanted in a heated greenhouse.
March 28 - April 10:	Tlansplanting 139 emerged individuals were transplanted to 5 inches clay pots and grown by ordinary manners in an unheated screen house.
May 9 to 23:	Pollination Pollen was collected from 121 blooming individuals to make a pollen mixture. The 644 flowers were pollinated with the pollen mixture.
June 12-23:	Collecting berries Berries (252) were collected. One month later, seeds were extracted from the harvested berries.

(III) Cultivation Test

[0021] Potato plants were planted in a culture tray (510 x 365 x 82 mm) at three different densities: As a "dense plot", 20 potato plants were planted per tray. As an "ordinary plot", 15 potato plants were planted per tray. As a "sparse plot", 8 potato plants were planted per tray. The density in the ordinary plot corresponds to that of the aforementioned selection tests. One test plot consists of 24 trays. The same cultivation test was repeated three times and the yields thereof were compared. The cultivation was performed as follows:

1996

[0022]

September 2:	Gibberellin treatment of the seeds Seeds obtained in the third selection were immersed in 2000 ppm gibberellin GA 3, overnight.
September 6:	Sowing seeds in cell trays
October 12-24:	Transplanting Individuals (3096) were planted in 216 culturing trays in total and grown by ordinary manners in an unheated glass house.

1997

[0023]

January 16-17:	Harvest of potatoes Potatoes were harvested independently in three densing plots.

[0024] In the aforementioned cultivation test, the yields (kg) of the potatoes per plot (24 culturing trays) which is classified by size were as follows. The size classification of the potato was made using sieves of 1.5 cm mesh and 4.0 cm mesh. More specifically, the potato passing through the 1.5-mesh sieve was regarded as an "extremely small potato". The potato remaining on the 4.0-mesh sieve was regarded as an "extremely large potato". A "mini-potato" was one which can pass through the 4.0-mesh sieve and remain on the 1.5-mesh sieve.

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		Potato Size		
District	Replicate	Mini-potato	Extremely large	Extremely small
High density plot	1	8.80	0.17	0.85
	2	8.80	0.19	0.72
	3	8.28	0.15	0.80
	Average	8.63	0.17	0.79
Ordinary plot	1	7.43	0.38	0.79
	2	7.46	0.21	0.92
	3	5.98	0.0	1.01
	Average	6.96	0.20	0.97
sparse plot	1	4.64	0.05	0.76
	2	5.36	0.20	0.36
	3	6.65	0.19	0.40
	Average	5.55	0.15	0.51

[0025] As is apparent from the aforementioned results, the yields of the mini-potato in the cultivation described above were about 80% or more. In particular, the mini-potato was obtained at a high yield in the dense plot.

[0026] According to the present invention as detailed in the foregoing, it is possible to obtain a mini-potato edible whole (including skin) in one bite. Therefore, a useful food material suitable for snack confectioneries and snacks taken with a beer can be provided.

Claims

1. A method of producing a bite-size mini-potato characterized by comprises the steps of:

   crossing Solanum stenotomum, Solanum phureja and Solanum goniocalyx, which are diploid potatoes native to the Andes, by open pollination, thereby forming a hybrid population rich in genetic variation;
   effecting breeding cultivation of the hybrid population and screening the hybrid population on the basis of selection criteria including a yield thereof, thereby reconstituting the hybrid population; and
   effecting seedling cultivation of the reconstituted hybrid population, thereby obtaining seeds, the seedling cultivation being effected under short-day light conditions while limiting a rhizosphere and controlling the growth at a slightly low temperature during a potato-tuber growing period.

2. The method according to claim 1, characterized in that a pot or a culture tray is used as means for limiting a rhizosphere.

3. The method according to claim 1, characterized in that the controlling of the growth during the potato-tuber growing period is made at a slightly lower temperature of 20 to 5°C, and preferably about 10°C.

4. The method according to claim 3, characterized in that the controlling the growth at a slightly lower temperature is performed in an unheated glass house or a vinyl-house.

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European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 98 12 0177

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
A	DATABASE FSTA INTERNATIONAL FOOD INFORMATION SERVICE (IFIS), FRANFURT/MAIN, DE QUIROS C F ET AL: "Biochemical and folk assessment of variability of Andean cultivated potatoes." XP002093712 * abstract * & ECONOMIC BOTANY, vol. 44, no. 2, 1990, pages 254-266, Dep. of Veg. Crops, Univ. of California, Davis, CA 95616, USA	1	A01H1/04 A01H3/02
A	WO 88 04137 A (NOVOTRADE R T) 16 June 1988 * claims *	1-4	
A	EP 0 574 283 A (COMMISSARIAT ENERGIE ATOMIQUE) 15 December 1993 * claims; figures *	1-4	
A	DATABASE CAB CAB INTERNATIONAL, WALLINGFORD, OXON, GB AN - 95:156499, RANALLI, P: "Microtuber and minituber production and field performance compared with normal tuber" XP002093713 * abstract * & POTATO RESEARCH, Vol. 37 No. 4, 1994, pages 383-391	1	TECHNICAL FIELDS SEARCHED (Int.Cl.6) A01H

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	17 February 1999	Fonts Cavestany, A

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

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ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 98 12 0177

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

17-02-1999

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 8804137 A	16-06-1988	AU 8337787 A	30-06-1988
		DK 426688 A	29-07-1988
		EP 0294412 A	14-12-1988
		FI 883607 A	01-08-1988
		JP 1502557 T	07-09-1989
EP 0574283 A	15-12-1993	FR 2691609 A	03-12-1993
		DE 69307603 D	06-03-1997
		DE 69307603 T	17-07-1997

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

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INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2010/050716

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01H1/02 A01H5/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, EMBL, WPI Data, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARIS B: "COMPARISON OF DIPLOID AND TETRAPLOID POTATO FAMILIES DERIVED FROM SOLANUM-PHUREJA X DIHAPLOID SOLANUM-TUBEROSUM HYBRIDS AND THEIR VEGETATIVELY DOUBLED COUNTERPARTS", EUPHYTICA, KLUWER ACADEMIC PUBLISHERS, NL, vol. 46, no. 1, 1 March 1990 (1990-03-01), pages 15-33, XP008132658, ISSN: 0014-2336, DOI: DOI:10.1007/BF00057615 table 8 ----- -/--	1-7,14, 15, 22-28, 31-33

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 March 2011	08/06/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bucka, Alexander

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL2010/050716

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-7, 32, 33(completely); 14, 15, 21-28, 31(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SHELLEY HERMUNDSTAD ET AL: "Tuber yield and tuber traits of haploid-wild species F1 hybrids", POTATO RESEARCH, ASSOCIATION FOR POTATO RESEARCH, WAGENINGEN, NL, vol. 29, 1 September 1986 (1986-09-01), pages 289-297, XP008132723, ISSN: 0014-3065, DOI: DOI:10.1007/BF02359958 page 290; figure 1	1-7,14, 15, 22-28, 31-33
X	WATANABE K N ET AL: "Generation of pest resistant, diploid potato germplasm with short-day adaptation from diverse genetic stocks", BREEDING SCIENCE, vol. 46, no. 4, 1996, pages 329-336, XP002577156, tables 1,2,8	1-7,14, 15, 22-28, 31-33
X	WANG D ET AL: "Improving main agronomic characters of dihaploids with 2n gametes by means of sexual hybridisation and anther culture of potato (Solanum tuberosum)", NEW ZEALAND JOURNAL OF CROP AND HORTICULTURAL SCIENCE, vol. 28, no. 1, March 2000 (2000-03), pages 1-8, XP002626196, ISSN: 0114-0671 table 3	1-7,14, 15, 22-28, 31-33
X	PHUMICHAI CHALERMPOL ET AL: "Toward the development of highly homozygous diploid potato lines using the self-compatibility controlling Sli gene", GENOME, vol. 48, no. 6, December 2005 (2005-12), pages 977-984, XP002577501, ISSN: 0831-2796	21
A	figure 1; table 1	1-7,14, 15, 22-28, 31-33

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BANI-AAMEUR F ET AL: "Enhancement of diploid Solanum chacoense Bitt. using adapted clones of Solanum phureja Juz. and Buk", EUPHYTICA, KLUWER ACADEMIC PUBLISHERS, NL, vol. 68, no. 3, 1 January 1993 (1993-01-01), pages 169-179, XP008132638, ISSN: 0014-2336, DOI: DOI:10.1007/BF00029870 figure 4	1-7,14, 15, 21-28, 31-33
A	EP 0 913 081 A1 (PRESIDENT OF KOBE UNIVERSITY A [JP]) 6 May 1999 (1999-05-06) page 6	1-7,14, 15, 21-28, 31-33
A	BUSO J A 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, vol. 122, no. 3, June 2003 (2003-06), pages 229-232, XP002626126, ISSN: 0179-9541 table 2	1-7,14, 15, 21-28, 31-33
A	VISKER M H P W ET AL: "Correlation between late blight resistance and foliage maturity type in potato", EUPHYTICA, vol. 137, no. 3, June 2004 (2004-06), pages 311-323, XP002577157, ISSN: 0014-2336 tables 1,2	1-7,14, 15, 21-28, 31-33
A	DINU I I ET AL: "Novel inter-series hybrids in Solanum, section Petota", THEORETICAL AND APPLIED GENETICS ; INTERNATIONAL JOURNAL OF PLANT BREEDING RESEARCH, SPRINGER, BERLIN, DE LNKD- DOI:10.1007/S00122-004-1782-X, vol. 110, no. 3, 1 February 2005 (2005-02-01), pages 403-415, XP019321811, ISSN: 1432-2242 page 409, column 410; table 2	1-7,14, 15, 21-28, 31-33

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JUAN PABLO RAIMONDI ET AL: "Crossability relationships among the wild diploid potato species Solanum kurtzianum, S. chacoense and S. ruiz-lealii from Argentina.", EUPHYTICA, vol. 132, no. 3, 2003, pages 287-295, XP002577155, ISSN: 0014-2336 tables 1,2,3	1-7,14, 15, 21-28, 31-33
A	EIJLANDER R ET AL: "Selection of vigorous and fertile S-homo- and heterozygous tester clones from self-incompatible diploid potato, Solanum tuberosum L", EUPHYTICA, vol. 97, no. 1, 1997, pages 97-111, XP002577160, ISSN: 0014-2336 table 1	1-7,14, 15, 21-28, 31-33
A	HOSAKA KAZUYOSHI ET AL: "Genetics of self-compatibility in a self-incompatible wild diploid potato species Solanum chacoense. 1. Detection of an S locus inhibitor (Sli) gene", EUPHYTICA, vol. 99, no. 3, 1998, pages 191-197, XP002577158, ISSN: 0014-2336 the whole document	1-7,14, 15, 21-28, 31-33
A	HERMSEN J G T ET AL: "INHERITANCE OF GENETIC MARKERS FROM 2 POTATO DI HA PLOIDS AND THEIR RESPECTIVE PARENT CULTIVARS", EUPHYTICA, KLUWER ACADEMIC PUBLISHERS, NL, vol. 27, no. 3, 1 January 1978 (1978-01-01), pages 681-688, XP008131098, ISSN: 0014-2336 page 681 - page 682; table 1	1-7,14, 15, 21-28, 31-33
A	DE MAINE M J: "The effects of inbreeding on the parental values of potato dihaploids", ANNALS OF APPLIED BIOLOGY, vol. 127, no. 1, 1995, pages 151-156, XP002626197, ISSN: 0003-4746 tables 1,2	1-7,14, 15, 21-28, 31-33

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International application No
PCT/NL2010/050716

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JANSKY S: "Overcoming hybridization barriers in potato", PLANT BREEDING, vol. 125, no. 1, February 2006 (2006-02), pages 1-12, XP002626127, ISSN: 0179-9541 the whole document	1-7,14, 15, 21-28, 31-33
A	SIMMONDS N W: "A review of potato propagation by means of seed, as distinct from clonal propagation by tubers", POTATO RESEARCH, vol. 40, 1997, pages 191-214, XP002575879, ISSN: 0014-3065 the whole document	1-7,14, 15, 21-28, 31-33

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/NL2010/050716

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0913081 A1	06-05-1999	CA 2252304 A1	30-04-1999
		DE 69810423 D1	06-02-2003
		DE 69810423 T2	02-10-2003
		JP 11127712 A	18-05-1999
		US 6084151 A	04-07-2000

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-7, 32, 33(completely); 14, 15, 21-28, 31(partially)

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.

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2. claims: 8-13, 29(completely); 14, 15, 21-28, 30, 31(partially)

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.

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3. claims: 16(completely); 17-20, 22-28, 30, 31(partially)

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; plants produced by this method.

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4. claim: 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.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P87313PC00	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/NL2010/050716	International filing date *(day/month/year)* 26.10.2010	Priority date *(day/month/year)* 26.10.2009
International Patent Classification (IPC) or national classification and IPC INV. A01H1/02		
Applicant Agventure B.V.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *(sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and/or sheets containing rectifications authorized by this Authority, unless those sheets were superseded or cancelled, and any accompanying letters (see Rules 46.5, 66.8, 70.16, 91.2, and Section 607 of the Administrative Instructions).
      ☐ sheets containing rectifications, where the decision was made by this Authority not to take them into account because they were not authorized by or notified to this Authority at the time when this Authority began to draw up this report, and any accompanying letters (Rules 66.4bis, 70.2(e), 70.16 and 91.2).
      ☐ superseded sheets and any accompanying letters, where this Authority either considers that the superseding sheets contain an amendment that goes beyond the disclosure in the international application as filed, or the superseding sheets were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in item 4 of Box No. I and the Supplemental Box (see Rule 70.16(b)).
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see paragraph 3*bis* of Annex C of the Administrative Instructions).

4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08.09.2011	13.02.2012
Name and mailing address of the international preliminary examining authority: European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Fax: +31 70 340 - 3016	Authorized officer Bucka, Alexander Telephone No. +31 70 340-2279

Agventure B.V. Ex. 2009
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Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a) and (b))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-67 as originally filed

Claims, Numbers

1-34 as originally filed

Drawings, Sheets

1/10-10/10 as originally filed

☒ a sequence listing - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since either they are considered to go beyond the disclosure as filed, or they were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in the Supplemental Box (Rules 70.2(c) and (c-bis)):
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:

5. ☐ This report has been established:
   - ☐ taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91 (Rules 66.1(d-bis) and 70.2(e)).
   - ☐ without taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91(Rules 66.4bis and 70.2(e)).

6. ☐ Supplementary international search report(s) from Authority(ies) has/have been received and taken into account in establishing this report (Rule 45bis.8(b) and (c)).

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 8-13, 16-20, 29, 30, 34(completely); 14, 15, 21-28, 31(partially)

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*.

   ☒ no international search report has been established for the said claims Nos. 8-13, 16-20, 29, 30, 34(completely); 14, 15, 21-28, 31(partially)

   ☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

      ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

      ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

      ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b) and 13*ter*.2.

   ☒ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-7, 14, 15, 21-28, 31-33
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-7, 14, 15, 21-28, 31-33
Industrial applicability (IA)	Yes:	Claims	1-7, 14, 15, 21-28, 31-33
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

   ☒ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search and/or examination

   ☐ to this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the sequence listing, which forms part of the basis of the report, may be marked "superseded."*

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

According to Rule 66.1(e) PCT, claims relating to inventions, in respect of which no international search report has been established, need not be the subject of international preliminary examination. Therefore, no Written Opinion of the International Searching Authority has been established for the subject matter of present claims 8-13, 16-20, 29, 30, 34 (completely), 14, 15, 21-28, 31 (partially), relating to inventions 2 to 4, which have not been searched due to a lack of unity of invention. The PCT International Search and Preliminary Examination Guidelines, 10.72, state that if "the applicant has not availed himself of the opportunity to have the international search report issued on at least some of the other inventions, this must be taken as an indication that the applicant is prepared for the international application to proceed on the basis that it relates to the invention first mentioned in the claims as originally contained in the international application as filed".

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Reference is made to the following documents:

D1 MARIS B: "COMPARISON OF DIPLOID AND TETRAPLOID POTATO FAMILIES DERIVED FROM SOLANUM-PHUREJA X DIHAPLOID SOLANUM-TUBEROSUM HYBRIDS AND THEIR VEGETATIVELY DOUBLED COUNTERPARTS", EUPHYTICA, KLUWER ACADEMIC PUBLISHERS, NL, vol. 46, no. 1, 1 March 1990 (1990-03-01), pages 15-33, XP008132658, ISSN: 0014-2336, DOI: DOI:10.1007/BF00057615

D2 SHELLEY HERMUNDSTAD ET AL: "Tuber yield and tuber traits of haploid-wild species F1 hybrids", POTATO RESEARCH, ASSOCIATION FOR POTATO RESEARCH, WAGENINGEN, NL, vol. 29, 1 September 1986 (1986-09-01), pages 289-297, XP008132723, ISSN: 0014-3065, DOI: DOI:10.1007/BF02359958

D3 WATANABE K N ET AL: "Generation of pest resistant, diploid potato germplasm with short-day adaptation from diverse genetic stocks", BREEDING SCIENCE, vol. 46, no. 4, 1996, pages 329-336, XP002577156,

D4 WANG D ET AL: "Improving main agronomic characters of dihaploids with 2n gametes by means of sexual hybridisation and anther culture of potato (Solanum tuberosum)", NEW ZEALAND JOURNAL OF CROP AND HORTICULTURAL SCIENCE, vol. 28, no. 1, March 2000 (2000-03), pages 1-8, XP002626196, ISSN: 0114-0671

D5 PHUMICHAI CHALERMPOL ET AL: "Toward the development of highly homozygous diploid potato lines using the self-compatibility controlling Sli gene", GENOME, vol. 48, no. 6, December 2005 (2005-12), pages 977-984, XP002577501, ISSN: 0831-2796

2 The present application appears to meet the criteria of Article 33(1) PCT, because the subject matter of **claims 1 to 7, 14, 15, 21 to 28 and 31 to 33** is new within the meaning of Article 33(2) PCT. While the prior art describes essentially homozygous, diploid, fertile potato plants with tuber yields of at least 200 g per plant (cf. D1, table 8; D2, page 290, "Results", figure 1; D3, table 8; D4, table 3), the cited documents do not test or characterise the self-compatibility of the described plants and therefore the self-compatible, diploid potato plants of claim 1 do not seem to be anticipated by the prior art.

3 D3, which could be regarded as the most relevant prior art for the subject matter of **claim 1**, describes essentially homozygous, diploid, fertile potato plants with tuber yields of at least 200 g per plant, from which the subject matter of claim 1 differs in that it requires the plants to be self-compatible.

The Applicant's attention is drawn to the fact that the claims do not require that the yield is obtained from potato plants grown from seedlings; hence, the plants described in the prior art meet the requirement of an average tuber yield of 200 grams per plant recited in claim 1. The plants of the prior art are likewise considered to be "essentially homozygous", which is defined as being at least 25% homozygous, when testing 100 loci (cf. description, page 21), i. e. the plants need only be homozygous in 25 loci.

The Applicant has contested that the plants described in D3 would meet the requirements of being "essentially homozygous" within the meaning of the description, since this required, according to the Applicant, the testing of 100 loci chosen *randomly* over the whole genome. However, the description, page 21, defines the term "essentially homozygous" as "a level of homozygosity of at least 25% ... homozygosity when testing 100, preferably 1000, more preferably at least 10.000 loci". The description does not require that these loci are chosen randomly. Therefore, the Applicant's argument concerning the homozygosity cannot convince.

Hence, in view of D3, the problem to be solved is the provision of further diploid, essentially homozygous, fertile potato plants with tuber yields of at least 200 g per plant.

The solution proposed by the subject matter of claim 1 consists of plants that are self-compatible.

The Applicant submitted that the problem was "to provide a diploid potato plant that is self-compatible and essentially homozygous and has a high tuber yield also from seedling plants". As already mentioned, the claims do not refer to yield from seedling plants. Since the plants of D3 have a yield of more than 200 g per plant (i. e. up to 600 g per plant; cf. D3, table 8), the yield recited in claim 1 is not a feature differing from the plants of the closest prior art. The same applies to the homozygosity, which is defined by the application as being homozygous in 25 loci (*supra*). Therefore, the only difference to the plants of D3 is their self-compatibility.

The advantages of self-compatible potato plants were *inter alia* known from D5 (cf. D5, page 978, left column), the teaching of which the skilled person would have been aware of, since it is a document from the same technical field. Starting from D3 and having regard of the teaching of D5, the skilled person would have considered the provision of self-compatible potato lines (cf. D5, the whole document). While D5 does not disclose the yield of the self-compatible potato plants, it appears to be self-evident that a breeder is interested in increasing the yield of any given crop. Therefore, the solution proposed by the present application cannot be considered to be inventive.

The same objections apply *mutatis mutandis* to the subject matter of **claims 2 to 7, 14, 15, 22 to 28 and 31 to 33.**

As far as the homozygosity is concerned, it is of course evident that once self-compatible lines are obtained (by following the teaching of D5), an increase in their homozygosity is a mere routine and readily achievable (cf. D5, table 2).

The Applicant has furthermore argued that there was a prejudice against breeding homozygous breeding lines, relying on a document from 1987 and an internet disclosure, both of which were however not submitted. Consequently, it is impossible to assess their teaching. However, a prejudice in the art is usually defined as an opinion widely or universally held by experts in the field, normally demonstrated by reference to reviews or encyclopedias. It appears that the existence of such a prejudice has not been sufficiently proven in the present case.

4 Claim 21 relates to deposited diploid, fertile, self-compatible potato plants with different degrees of homozygosity. Their yield is characterised as intermediate/high, although exact data are not disclosed in the application.

Document D5, which is considered to represent the most relevant state of the art for the subject matter of claim 21, discloses (cf. D5, figure 1, table 1) diploid, fertile, self-compatible, homozygous potato plants (inbred series B), from which the subject matter of claim 21 differs in that provides apparently genetically different diploid potato plants.

The problem to be solved by the present invention may therefore be regarded as the provision of further diploid potato plants.

The solution proposed in **claim 21** of the present application cannot be considered as involving an inventive step (Article 33(3) PCT), since the skilled person obviously would have considered the provision of further diploid homozygous self-compatible potato lines based e. g. on the teaching of document D5 (cf. D5, figure 1, table 1). It is emphasized that the yield of the deposited lines is not quantitated and that some lines are only 25% homozygous. Hence, the Applicant's arguments concerning the yield and the homozygosity, submitted in reply to the Written Opinion of the International Searching Authority, do not appear to apply to (some of) the deposited lines. The deposited lines do not appear to be associated with any unexpected or surprising technical effect.

5 According to the PCT Guidelines, A5.26[1], claims for products defined in terms of a process of manufacture (cf. **claims 14, 15, 22 to 28, 31, 33**) are to be construed as claims to the product as such. A product is not rendered novel merely by the fact that it is produced by means of a new process.

6 The attention of the Applicant is drawn to the fact that the application may not be amended in such a way that it contains subject matter which extends beyond the content of the application as filed (Article 34(2)(b) PCT).

Electronic Acknowledgement Receipt

EFS ID:	12836045
Application Number:	13504156
International Application Number:	
Confirmation Number:	6337
Title of Invention:	Hybrid Seed Potato Breeding
First Named Inventor/Applicant Name:	Willem Hendrik Lindhout
Customer Number:	22910
Filer:	John P. Iwanicki/Graci Smith
Filer Authorized By:	John P. Iwanicki
Attorney Docket Number:	05032-00255
Receipt Date:	22-MAY-2012
Filing Date:	
Time Stamp:	14:55:04
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	IDS.pdf	613920 8cce959ac8190c3ceccbf0de54ac73a42a479513	no	5

Warnings:	
Information:	

Agventure B.V. Ex. 2009
IPR2025-00136
Page 354

A U.S. Patent Number Citation or a U.S. Publication Number Citation is required in the Information Disclosure Statement (IDS) form for autoloading of data into USPTO systems. You may remove the form to add the required data in order to correct the Informational Message if you are citing U.S. References. If you chose not to include U.S. References, the image of the form will be processed and be made available within the Image File Wrapper (IFW) system. However, no data will be extracted from this form. Any additional data such as Foreign Patent Documents or Non Patent Literature will be manually reviewed and keyed into USPTO systems.

2	Foreign Reference	EP09130813.pdf	62996 524615225bd2e82b177220d6f0e94a3a3b8c538f	no	8
Warnings:					
Information:					
3	Non Patent Literature	ISR.pdf	51704 df8e7642ca816988a3628bbd19d2fe7dda244873	no	9
Warnings:					
Information:					
4	Non Patent Literature	IPRP.pdf	98498 a930887330e2a52876e8690d28d32548ebe28e26	no	9
Warnings:					
Information:					
5	Non Patent Literature	Bani_Aameur.pdf	2227728 df1b3d5294fa52ce4d9dd679eff571852f81509e	no	11
Warnings:					
Information:					
6	Non Patent Literature	Buso.pdf	853913 1092ac63c85ef3cde03872be91c8bd244c13978f	no	4
Warnings:					
Information:					
7	Non Patent Literature	DeMaine.pdf	905739 cdfc3c8178a30b01b16970d1cec57ab31003b615	no	6
Warnings:					
Information:					
8	Non Patent Literature	Dinu.pdf	2632924 f7bf87408cdc8785c8871fe158561c162de0892f	no	13
Warnings:					
Information:					
9	Non Patent Literature	Eijlander.pdf	2314871 cc75eaf57759eca6c9ad9c5ffa74397a13fffad3	no	15
Warnings:					
Information:					

10	Non Patent Literature	Hermsen.pdf	1717507 73ca60302a15b92e75ec5d304bad8a44fe25efee	no	12
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Information:					
11	Non Patent Literature	Hermunstad.pdf	1591190 0cd34a2be4ceca6f44e13b5a864359fa4ec1c1e4	no	9
Warnings:					
Information:					
12	Non Patent Literature	Hosaka.pdf	1044670 582dbf7402cef1777b9069c790ed0439c985045c	no	7
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Information:					
13	Non Patent Literature	Jansky.pdf	2796366 1fe6870cd0122eea7e4bc2da96d5858b0cf2a184	no	12
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Information:					
14	Non Patent Literature	Maris.pdf	3408067 d7dd2fede5c3648f627bd8e06beeb686bafd46c5	no	19
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Information:					
15	Non Patent Literature	Pablo.pdf	1431646 db9ddeee021e08ac358e108f0ccdc261d953842f	no	9
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Information:					
16	Non Patent Literature	Phumichai.pdf	1467290 51c27072833153cd2b210a947d6ac2a881c93f52	no	8
Warnings:					
Information:					
17	Non Patent Literature	Simmonds.pdf	3568536 56d97f6f58075127f5cb42a0affa27ee7be0bd11	no	24
Warnings:					
Information:					
18	Non Patent Literature	Visker.pdf	2079546 3e68cc88f25810e9b9a54de8baad7cc9d7c7497f	no	13
Warnings:					
Information:					

19	Non Patent Literature	Wang.pdf	1405174 b1d26b0eee30d769077d0bcca190a00472f00459	no	8
Warnings:					
Information:					
20	Non Patent Literature	Watanabe.pdf	1661388 717a107792d0feb3db46968887fbbad1deaf4b18	no	9
Warnings:					
Information:					
			Total Files Size (in bytes):	31933673	

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.

PATENT
ATTORNEY DOCKET NO. 005032-00255

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Willem Hendrik Lindhout, et al.	)	Examiner:
	)	TBA
Serial No.: 13/504,156	)	
	)	Art Unit: TBA
Filed: April 26, 2012	)	
	)	Conf. No.: 6337
Title: HYBRID SEED POTATO BREEDING	)	

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

PRELIMINARY AMENDMENT
AND SUBMISSION OF SEQUENCE LISTING

Dear Sir:

Preliminary to examination, please enter the following amendments in the above-referenced application.

Amendments to the specification begin at page 2.

Remarks are at page 6.

Amendment to the Specification:

At page 53 of the Specification please amend Table 5A as follows:

Table 5A. Sequences of 100 SNP markers in potato

SNPs

SNPID	SNPNum	AlleleY	AlleleX	Sequence	Sequence Identifier
				ACCTGGTGCAGGCCT[C/G]	SEQ ID NO:1
PotSNP002	11940065	G	C	CGTATTGATACGATT	
				GACTGTGATTCGAAA[T/C]	SEQ ID NO:2
PotSNP011	11940098	C	T	GTTTACCTATCTCTT	
				TCGTAGAAATGACAT[T/C]	SEQ ID NO:3
PotSNP021	11940075	C	T	TGGGGTGCTAGAGAT	
				AGTTTCCAGCTGATG[T/C]	SEQ ID NO:4
PotSNP026	11940044	C	T	TATTCTAGGAGACGA	
				AGAGGCTCTTGAGGC[A/G]	SEQ ID NO:5
PotSNP032	11940037	G	A	CAGCGAAAAGAGGAA	
				TGGTGACGTTCATGT[T/C]	SEQ ID NO:6
PotSNp038	11940016	C	T	GGACGTAATGCTTCT	
				AGTATCTCCGTCCGA[A/C]	SEQ ID NO:7
PotSNP043	11940036	C	A	TCACAGTCCTACTCT	
				GGTGCTAAGATTCGC[A/G]	SEQ ID NO:8
PotSNP045	11940094	G	A	TTGATGATTCACTAG	
				CCAACACTGGAAAAA[T/C]	SEQ ID NO:9
PotSNP047	11940054	C	T	TATGGAAGCAGAAAG	
				GCTAGCATTAGAGCT[T/C]	SEQ ID NO:10
PotSNP052	11940097	C	T	GCTAAGCGACTCAAT	
				GCCTTGGAGTAAGAG[A/G]	SEQ ID NO:11
PotSNP055	11940021	G	A	TTTTCTGTTTTGGGC	
				GGATGTTCATCAACG[T/C]	SEQ ID NO:12
PotSNP057	11940040	C	T	CGACTTCGACAAGCT	
				ACAACCTGCAAGAGC[T/C]	SEQ ID NO:13
PotSNP061	11940032	C	T	TTGCATCTGTTTAGT	
				TACAATCATGATCCT[T/C]	SEQ ID NO:14
PotSNP068	11940023	C	T	TTGCCTAATTGCCCT	
				CTGAGCTAGTAGATC[A/G]	SEQ ID NO:15
PotSNP072	11940005	G	A	ATCAAACGGTGCTCC	
				GACTGTATGGACAGT[T/G]	SEQ ID NO:16
PotSNP073	11940076	G	T	ATTGGGTCAGAGGAC	
				AAGATGACGTGGAGT[T/C]	SEQ ID NO:17
PotSNP080	11940068	C	T	CGGCACTGCGCCGTT	
				GCCGCACAAGGTGCT[T/C]	SEQ ID NO:18
PotSNP081	11940047	C	T	GCCGTCGATGTTAGC	
				AGGTAGTGTTGCAAA[T/C]	SEQ ID NO:19
PotSNP083	11940033	C	T	ACAATTAGAGGGTTG	
				AAGTACATTTATGAG[A/G]	SEQ ID NO:20
PotSNP089	11940062	G	A	AGATTAACAAGTGTT	
				ACTGCTGGAACTGAG[T/C]	SEQ ID NO:21
PotSNP099	11940092	C	T	TGAACAATGCTATTA	
				GAGTGCCGGCAGCGT[T/C]	SEQ ID NO:22
PotSNP100	11940083	C	T	ACAGATCCGACGATG	
				TATGGCAGAGAAAGA[A/G]	SEQ ID NO:23
PotSNP102	11940070	G	A	ATGGAGTATAGAGTG	
PotSNP1026	11940059	G	C	TAAACTCCAACATAT[C/G]	SEQ ID NO:24

				AACACAAATTAGTGC	
				TTCTACATCCTTCGC[A/G]	SEQ ID NO:25
PotSNP1034	11940001	G	A	GGCATTTTTGAGGAG	
				TTTGGGGAGGAACAC[C/T]	SEQ ID NO:26
PotSNP1049	11940051	T	C	TGTGCTTTAGAACTC	
				TGTCACTAAGAAAGC[C/A]	SEQ ID NO:27
PotSNP1057	11940022	A	C	CAAGAGGCAATGCAG	
				GCAAGCTACTTGTTC[T/G]	SEQ ID NO:28
PotSNP106	11940010	G	T	AAAGGACCTGCTACT	
				AGCGAGTTTGGATGC[T/G]	SEQ ID NO:29
PotSNP1072	11940027	G	T	GAGCTTTTGCAGTTA	
				TACAAAGTTCACTGC[T/C]	SEQ ID NO:30
PotSNP1083	11940019	C	T	GAGGAGGTCATGCAG	
				GATGAGGAAAGGCAC[C/A]	SEQ ID NO:31
PotSNP1105	11940041	A	C	GGCGGAGGTGTTGGA	
				CACCACCGTCCAAAC[C/T]	SEQ ID NO:32
PotSNP1115	11940071	T	C	GGTTTTGCCAATCGC	
				GGTCCCTGAGCAAGG[C/T]	SEQ ID NO:33
PotSNP1122	11940060	T	C	AATCAACGAATTAGG	
				AGTCTCTACAAACCC[A/G]	SEQ ID NO:34
PotSNP1139	11940004	G	A	TGGTTTCGATCTTGA	
				TAATGTGGTCGGCAC[A/G]	SEQ ID NO:35
PotSNP116	11940058	G	A	CTGACGCTTGCTGAT	
				AGATGCATTCTATGC[A/G]	SEQ ID NO:36
PotSNP118	11940091	G	A	GAACCACAAAGATAT	
				TTCGTGTTGCACTCA[A/G]	SEQ ID NO:37
PotSNP120	11940082	G	A	CGATTCAGTTGGATG	
				AGCTTGTGCTAATAG[T/C]	SEQ ID NO:38
PotSNP121	11940099	C	T	TGTGCTGATTTTCTC	
				TATGCAATCCCAGCC[A/G]	SEQ ID NO:39
PotSNP122	11940078	G	A	ACATATCCGATCCAT	
				TCATACTTCACATAT[A/G]	SEQ ID NO:40
PotSNP124	11940055	G	A	TCAGAGTGGTCTGAT	
				AGAGAAAAAAGCTGT[T/C]	SEQ ID NO:41
PotSNP130	11940050	C	T	GTTGTTGATGACGAG	
				CGCGTATTTCCCTTA[T/C]	SEQ ID NO:42
PotSNP134	11940080	C	T	TATAACTCGTTCTCT	
				TTACATATGCAATAG[T/C]	SEQ ID NO:43
PotSNP138	11940052	C	T	GGTGGTGATCTTTAT	
				GAAGTTGTCTTACAT[T/C]	SEQ ID NO:44
PotSNP152	11940077	C	T	GCTCTTGACTATGAA	
				CTATGATGCATTTAG[T/C]	SEQ ID NO:45
PotSNP162	11940074	C	T	TCATGGAGCAAACTT	
				AGTTCCTTTTTCTCC[A/G]	SEQ ID NO:46
PotSNP165	11940009	G	A	AGTTCTCAGACTCCG	
				GAGGAATTGATGCGT[T/G]	SEQ ID NO:47
PotSNP178	11940026	G	T	CTGAACTGGCACAGT	
				ATACACAGGAAGGGT[A/G]	SEQ ID NO:48
PotSNP182	11940095	G	A	AAGTGCTTCAAATTG	
				TTTGACCATTCTTTC[A/G]	SEQ ID NO:49
PotSNP184	11940038	G	A	GTCCTAGCTAACCAC	
				AAGATAAGAAGAAAG[T/C]	SEQ ID NO:50
PotSNP185	11940002	C	T	TAAGAGAACCAAGAT	
				GGCCCACTTTCCACA[T/C]	SEQ ID NO:51
PotSNP194	11940064	C	T	ATTCAATTTGGTCTC	
PotSNP205	11940042	G	A	TCAACTCACCACTGG[A/G]	SEQ ID NO:52

				TTTTACTCAAAATCA	
				TAATATGTTGTGTCT[A/G]	SEQ ID NO:53
PotSNP213	11940096	G	A	TAATTTTTGGGTCAT	
				CATTAACCAAGGTTG[T/C]	SEQ ID NO:54
PotSNP220	11940013	C	T	ATCGTTCGAGAGATC	
				TCCTTTGTCATTGTA[T/C]	SEQ ID NO:55
PotSNP229	11940056	C	T	GCCATGGAAGAAGCA	
				GCTTTGTTTAAGAAC[A/T]	SEQ ID NO:56
PotSNP392	11940003	T	A	GCCAGAAGAAGTTGC	
				GCCTACGCGGCATGA[A/G]	SEQ ID NO:57
PotSNP401	11940012	G	A	GATGTGGATATTGTT	
				AAAGCTCAAATTAAA[C/T]	SEQ ID NO:58
PotSNP402	11940072	T	C	GACGATGTTCAGGAA	
				GGCCAACCAATTCCA[G/A]	SEQ ID NO:59
PotSNP423	11940020	A	G	CGTCTTCTCCCAGAC	
				AAAACCATTTGGTCT[T/C]	SEQ ID NO:60
PotSNP446	11940081	C	T	GTTGATAATTCTGAG	
				TACATAGCTCTACAA[G/A]	SEQ ID NO:61
PotSNP458	11940069	A	G	CTTGGAAACATGTCA	
				GACACAAAATTCATA[A/T]	SEQ ID NO:62
PotSNP470	11940043	T	A	TGGCTGATACTTTGT	
				GCAACTTGTCGTCGG[A/T]	SEQ ID NO:63
PotSNP543	11940049	T	A	AAACTCGACTAGATT	
				CTAGAAACAAAATCA[C/A]	SEQ ID NO:64
PotSNP567	11940014	A	C	AATATAAGTTACATA	
				GTTGTTTGCTTTGTT[A/G]	SEQ ID NO:65
PotSNP569	11940028	G	A	TGCTGACTTATTGTA	
				ATGCTTCTTGGTTGC[T/C]	SEQ ID NO:66
PotSNP573	11940034	C	T	GTTTGTTTAGGCTCT	
				AAATATGTTGTTCCA[C/T]	SEQ ID NO:67
PotSNP580	11940087	T	C	TAGAAAAAAGAGTAA	
				GTGAATACTGGTATC[T/C]	SEQ ID NO:68
PotSNP586	11940088	C	T	TTCCAATTGAAGTCA	
				AATATTCTTGTAGTA[T/C]	SEQ ID NO:69
PotSNP587	11940067	C	T	ATATTGTCTAAAATC	
				CAACGATATCGAGAA[C/T]	SEQ ID NO:70
PotSNP607	11940084	T	C	TATTGTTCTAAGGGA	
				GCTGAAAGTAAAGAC[A/G]	SEQ ID NO:71
PotSNP626	11940045	G	A	GTGTTCAACATCTTG	
				CATGCAATTGTAATA[G/T]	SEQ ID NO:72
PotSNP645	11940007	T	G	TATCAATGTTTGTTG	
				TGCTCAAGCTGCCAA[C/T]	SEQ ID NO:73
PotSNP652	11940024	T	C	GCTAATCCGCTTTAC	
				CAAGATATATGCAGC[A/G]	SEQ ID NO:74
PotSNP700	11940061	G	A	GAGTCCCTTCGAGCA	
				TTATGGTTATGGTGG[T/A]	SEQ ID NO:75
PotSNP702	11940039	A	T	GGAGGTTGGATTAAT	
				GGGAGGGTGAAAATA[G/C]	SEQ ID NO:76
PotSNP712	11940057	C	G	TCGAAAACGGGCAAC	
				GATTGTTATCCTTCC[A/G]	SEQ ID NO:77
PotSNP713	11940073	G	A	CCACCTCCAACAGAT	
				CAACTTCCAGTCGTG[G/A]	SEQ ID NO:78
PotSNP731	11940035	A	G	GAGTGGGGTTAACGG	
				ATGGTGTCTCCAAGG[A/G]	SEQ ID NO:79
PotSNP753	11940046	G	A	ACTATTACAGTCACT	
PotSNP759	11940090	C	T	AACATCGATAGGACT[T/C]	SEQ ID NO:80

				TGAAGGCCGCGGATG	
				CTGCCACTGCGGCTG[C/T]	SEQ ID NO:81
PotSNP766	11940089	T	C	AGGTTCTGCAGCTGC	
				TGAAATGATTGGATT[A/G]	SEQ ID NO:82
PotSNP775	11940079	G	A	TGACCCATAGTTTAG	
				ATCTCTAAGTTGGGG[A/C]	SEQ ID NO:83
PotSNP796	11940025	C	A	AACCCATAATTTCTA	
				CGCTTGGGAAGAACC[A/T]	SEQ ID NO:84
PotSNP827	11940053	T	A	TTCGGTCCAGTTTTG	
				CCAATTCCGACGGCC[C/T]	SEQ ID NO:85
PotSNP834	11940006	T	C	TCGTCGTTTCACCGC	
				GGTCGACCATTGGGG[C/A]	SEQ ID NO:86
PotSNP853	11940066	A	C	CCCGTAGAGGGTATA	
				AAGGCGAATTCCGGT[T/G]	SEQ ID NO:87
PotSNP866	11940048	G	T	CCCGAAATGGATCTC	
				TCATTACATCAAAAT[G/A]	SEQ ID NO:88
PotSNP893	11940011	A	G	TTTTATCAATGCCCA	
				TGATCCATTTGGTCT[A/T]	SEQ ID NO:89
PotSNP908	11940100	T	A	GACCAGTTCTTGACA	
				TGCTGTGCTCCTTCT[A/T]	SEQ ID NO:90
PotSNP910	11940017	T	A	TTCCTCATCTCTCAC	
				TCATTCTCACTCTCC[T/A]	SEQ ID NO:91
PotSNP928	11940030	A	T	GCTGAACAAGCCATT	
				AAAAGACAAGAATCT[G/T]	SEQ ID NO:92
PotSNP947	11940015	T	G	GTTCAGATTCTCCAG	
				TGGGGAAAACTTAAT[A/T]	SEQ ID NO:93
PotSNP948	11940063	T	A	TGACAGCCAAGGCGT	
				GGTTTTGTGGACTGC[T/C]	SEQ ID NO:94
PotSNP960	11940031	C	T	AACACTGAAAGATAC	
				TTATTTCAATGAAGC[T/A]	SEQ ID NO:95
PotSNP964	11940029	A	T	TCTGGTGGACGTTAT	
				TCTGTGGTTTTTTCA[T/C]	SEQ ID NO:96
PotSNP982	11940085	C	T	CACCTCCATCTTCAA	
				TAAGATTAGTTCTAA[A/G]	SEQ ID NO:97
PotSNP983	11940086	G	A	CTTAGCGCAGAAGAC	
				GAGCTCTATAAGTGC[G/A]	SEQ ID NO:98
PotSNP985	11940018	A	G	CCTGTTACTCCACCT	
				GGGCTATGGTTGGTT[A/C]	SEQ ID NO:99
PotSNP987	11940008	C	A	GGAAAAAAGGTGCAA	
				GGATATTGGGTTCGT[T/C]	SEQ ID NO:100
PotSNP996	11940093	C	T	CCATGATTCGTGGCA	

REMARKS

The application has been amended to include sequence identifiers. The amendments do not add new matter. The application has been further amended to incorporate by reference a computer readable Squence Listing, which Squence Listing accompanies this amendment in both written form and in ANSI text format, as the file name "05032_00255_ST25.txt." The sequence listing does not contain matter which goes beyond the disclosure in the application as filed.

I hereby state that the Squence Listing information recorded in computer readable form, which is being submitted herewith in the above-identified application, is identical to the written copy of the Squence Listing.

Prior to examination, Applicant respectfully requests entry of the above amendments. The Commissioner is hereby authorized to charge any additional fees or credit overpayment to Deposit Account No. 19-0733.

Respectfully submitted,

Date: July 10, 2012

John P. Iwanicki, Reg. No. 34,628
BANNER & WITCOFF, LTD.
28 State Street, Suite 1800
Boston, MA 02109
Telephone: (617) 720-9600

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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(s), I/we declare that:

This declaration is directed to:

- ☐ The attached application, or
- ☑ United States application or PCT international application number 13/504,156 filed on April 26, 2012
- ☐ As amended on _____ (if applicable);

I/we believe that I/we am/are the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought;

I/we have reviewed and understand the contents of the above-identified application, including the claims, as amended by any amendment specifically referred to above;

I/we acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me/us to be material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT International filing date of the continuation-in-part application.

WARNING:

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.

All statements made herein of my/our own knowledge are true, all statements made herein on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and may jeopardize the validity of the application or any patent issuing thereon.

FULL NAME OF INVENTOR(S)

Inventor one: Willem Hendrik Lindhout Date: 30 May 2012

Signature: W Lindhout Citizen of: NL

Inventor two: Teunis Philippus Schotte Date: 30 May 2012

Signature: _____ Citizen of: NL

☑ Additional inventors or a legal representative are being named on 2 additional form(s) attached hereto.

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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

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- ☑ United States application or PCT international application number 13/504,156
  filed on April 26, 2012
  - ☐ As amended on _____ (if applicable);

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I/we have reviewed and understand the contents of the above-identified application, including the claims, as amended by any amendment specifically referred to above;

I/we acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me/us to be material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT International filing date of the continuation-in-part application.

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FULL NAME OF INVENTOR(S)

Inventor one: Richard Gerardus Franciscus Visser Date: 29 May 2012

Signature: R.G.F. Visser Citizen of: NL

Inventor two: Herman Johannes van Eck Date: 23 May 2012

Signature: Citizen of: NL

☐ Additional inventors or a legal representative are being named on _____ additional form(s) attached hereto.

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Title of Invention	Hybrid Seed Potato Breeding

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This declaration is directed to:

- ☐ The attached application, or
- ☑ United States application or PCT international application number 13/504,156 filed on April 26, 2012
- ☐ As amended on _____ (if applicable);

I/we believe that I/we am/are the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought;

I/we have reviewed and understand the contents of the above-identified application, including the claims, as amended by any amendment specifically referred to above;

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All statements made herein of my/our own knowledge are true, all statements made herein on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and may jeopardize the validity of the application or any patent issuing thereon.

FULL NAME OF INVENTOR(S)

Inventor one: Ronaldus Cornelis Bernardus Hutten Date: 23 MAY 2012

Signature: _____ Citizen of: NL

Inventor two: _____ Date: _____

Signature: _____ Citizen of: _____

☐ Additional inventors or a legal representative are being named on _____ additional form(s) attached hereto.

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POWER OF ATTORNEY OR REVOCATION OF POWER OF ATTORNEY WITH A NEW POWER OF ATTORNEY AND CHANGE OF CORRESPONDENCE ADDRESS

Application Number	13/504,156
Filing Date	2012-04-26
First Named Inventor	Willem Hendrik Lindhout
Title	Hybrid Seed Potato Breeding
Art Unit	TBA
Examiner Name	TBA
Attorney Docket Number	05032-00255

I hereby revoke all previous powers of attorney given in the above-identified application.

☐ A Power of Attorney is submitted herewith.

OR

☒ I hereby appoint Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith: 22910

OR

☐ I hereby appoint Practitioner(s) named below as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

Practitioner(s) Name	Registration Number

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☐ The address associated with Customer Number:

OR

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Country					
Telephone		Email			

I am the:

☒ Applicant/Inventor.

OR

☐ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) (Form PTO/SB/96) submitted herewith or filed on _____.

SIGNATURE of Applicant or Assignee of Record

Signature	Willem Hendrik Lindhout	Date	30 May 2012
Name	Willem Hendrik Lindhout	Telephone	
Title and Company	Inventor		

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☒ *Total of 5 forms are submitted.

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Country					
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OR

☐ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) (Form PTO/SB/96) submitted herewith or filed on _____

SIGNATURE of Applicant or Assignee of Record

Signature		Date	30 May 2012
Name	Teunis Philippus Schotte	Telephone	
Title and Company	Inventor		

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SIGNATURE of Applicant or Assignee of Record

Signature	R.G.F. Visser	Date	29 May 2012
Name	Richard Gerardus Franciscus Visser	Telephone	
Title and Company	Inventor		

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☒ *Total of 5 forms are submitted.

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 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 3 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.**

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POWER OF ATTORNEY OR REVOCATION OF POWER OF ATTORNEY WITH A NEW POWER OF ATTORNEY AND CHANGE OF CORRESPONDENCE ADDRESS

Application Number	13/504,156
Filing Date	2012-04-26
First Named Inventor	Willem Hendrik Lindhout
Title	Hybrid Seed Potato Breeding
Art Unit	TBA
Examiner Name	TBA
Attorney Docket Number	05032-00255

I hereby revoke all previous powers of attorney given in the above-identified application.

☐ A Power of Attorney is submitted herewith.

OR

☒ I hereby appoint Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith: 22910

OR

☐ I hereby appoint Practitioner(s) named below as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

Practitioner(s) Name	Registration Number

Please recognize or change the correspondence address for the above-identified application to:

☒ The address associated with the above-mentioned Customer Number.

OR

☐ The address associated with Customer Number:

OR

☐ Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the:

☒ Applicant/Inventor.

OR

☐ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) (Form PTO/SB/96) submitted herewith or filed on _____

SIGNATURE of Applicant or Assignee of Record

Signature		Date	23 MAY 2012
Name	Herman Johannes van Eck	Telephone	
Title and Company	Inventor		

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☒ *Total of 5 forms are submitted.

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Application Number	13/504,156
Filing Date	2012-04-26
First Named Inventor	Willem Hendrik Lindhout
Title	Hybrid Seed Potato Breeding
Art Unit	TBA
Examiner Name	TBA
Attorney Docket Number	05032-00255

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Practitioner(s) Name	Registration Number

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OR

☐ The address associated with Customer Number:

OR

☐ Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the:

☒ Applicant/Inventor.

OR

☐ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) (Form PTO/SB/96) submitted herewith or filed on _____.

SIGNATURE of Applicant or Assignee of Record

Signature		Date	23 MAY 2012
Name	Ronaldus Cornelis Bernardus Hutten	Telephone	
Title and Company	Inventor		

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☒ *Total of 5 forms are submitted.

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

Electronic Patent Application Fee Transmittal

Application Number:	13504156
Filing Date:	
Title of Invention:	Hybrid Seed Potato Breeding
First Named Inventor/Applicant Name:	Willem Hendrik Lindhout
Filer:	John P. Iwanicki/Diana Freitas
Attorney Docket Number:	05032-00255

Filed as Small Entity

U.S. National Stage under 35 USC 371 Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Oath/decl > 30 mo. from priority date	2617	1	65	65
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

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IPR2025-00136
Page 372

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	**65**

Electronic Acknowledgement Receipt

EFS ID:	13211155
Application Number:	13504156
International Application Number:	
Confirmation Number:	6337
Title of Invention:	Hybrid Seed Potato Breeding
First Named Inventor/Applicant Name:	Willem Hendrik Lindhout
Customer Number:	22910
Filer:	John P. Iwanicki/Diana Freitas
Filer Authorized By:	John P. Iwanicki
Attorney Docket Number:	05032-00255
Receipt Date:	10-JUL-2012
Filing Date:	
Time Stamp:	11:32:28
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$65
RAM confirmation Number	9836
Deposit Account	190733
Authorized User	

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

Charge any Additional Fees required under 37 C.F.R. 1.492 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)					
File Listing:					
Document Number	**Document Description**	**File Name**	**File Size(Bytes)/ Message Digest**	**Multi Part /.zip**	**Pages (if appl.)**
1		Preliminary_Amendment_and_Submission_of_Squence_Listing.pdf	268794 8751a098eda3b6555490004deef04fa682994231	yes	6
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Preliminary Amendment		1	1	
	Specification		2	5	
	Applicant Arguments/Remarks Made in an Amendment		6	6	
Warnings:					
Information:					
2	Sequence Listing (Text File)	05032-00255_SeqList_ST25.txt	21902	no	0
Warnings:					
Information:					
3	Documents submitted with 371 Applications	Declaration.pdf	360648 2cc2f380c1ce3b1c94664131f9e0932e32640f0b	no	3
Warnings:					
Information:					
4	Documents submitted with 371 Applications	Power_of_Attorney.pdf	505659 61c2df5b0e5a802ac5c0c99e62a5a8964a1f7230	no	5
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	29828 c4cc85c72cefb0f88551b485a9586c897f33a7a8	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1186831		

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.

Electronic Acknowledgement Receipt

EFS ID:	13211155
Application Number:	13504156
International Application Number:	
Confirmation Number:	6337
Title of Invention:	Hybrid Seed Potato Breeding
First Named Inventor/Applicant Name:	Willem Hendrik Lindhout
Customer Number:	22910
Filer:	John P. Iwanicki/Diana Freitas
Filer Authorized By:	John P. Iwanicki
Attorney Docket Number:	05032-00255
Receipt Date:	10-JUL-2012
Filing Date:	
Time Stamp:	11:32:28
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$65
RAM confirmation Number	9836
Deposit Account	190733
Authorized User	

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

Charge any Additional Fees required under 37 C.F.R. 1.492 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)					
File Listing:					
Document Number	**Document Description**	**File Name**	**File Size(Bytes)/ Message Digest**	**Multi Part /.zip**	**Pages (if appl.)**
1		Preliminary_Amendment_and_Submission_of_Squence_Listing.pdf	268794 8751a098eda3b6555490004deef04fa682994231	yes	6
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Preliminary Amendment		1	1	
	Specification		2	5	
	Applicant Arguments/Remarks Made in an Amendment		6	6	
Warnings:					
Information:					
2	Sequence Listing (Text File)	05032-00255_SeqList_ST25.txt	21902	no	0
Warnings:					
Information:					
3	Documents submitted with 371 Applications	Declaration.pdf	360648 2cc2f380c1ce3b1c94664131f9e0932e32640f0b	no	3
Warnings:					
Information:					
4	Documents submitted with 371 Applications	Power_of_Attorney.pdf	505659 61c2df5b0e5a802ac5c0c99e62a5a8964a1f7230	no	5
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	29828 c4cc85c72cefb0f88551b485a9586c897f33a7a8	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1186831		

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.

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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.

DocCode – SEQ.TXT

SCORE Placeholder Sheet for IFW Content

Application Number: 13504156 Document Date: 07/10/2012

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

- Sequence Listing

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

To access the documents in the SCORE database, refer to instructions developed by SIRA.

At the time of document entry (noted above):

- Examiners may access SCORE content via the eDAN interface.
- Other USPTO employees can bookmark the current SCORE URL (http://es/ScoreAccessWeb/).
- External customers may access SCORE content via the Public and Private PAIR interfaces.

Form Revision Date: February 8, 2006

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IPR2025-00136
Page 380

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number	Filing Date	☐ To be Mailed
13/504,156	07/10/2012	

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			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 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).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
			TOTAL			TOTAL	

* If the difference in column 1 is less than zero, enter "0" in column 2.

APPLICATION AS AMENDED – PART II

AMENDMENT 07/10/2012

	(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 ($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	* 34	Minus	** 34	= 0	X $30 =	0	OR	X $ =	
Independent (37 CFR 1.16(h))	* 7	Minus	***7	= 0	X $125 =	0	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	0	OR	TOTAL ADD'L FEE	

AMENDMENT

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)	OR	RATE ($)	ADDITIONAL FEE ($)
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 number found in the appropriate box in column 1.

Legal Instrument Examiner:
/ANDREW j1339 JAMES JR/

This collection of information is required by 37 CFR 1.16. 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 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.**

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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number	Filing Date	☐ To be Mailed
13/504,156	07/10/2012	

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			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 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).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	07/10/2012	(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 ($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 27	Minus	**	= 27	X $30 =	810	OR	X $ =	
	Independent (37 CFR 1.16(h))	* 7	Minus	***	= 7	X $125 =	875	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	1685	OR	TOTAL ADD'L FEE	

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)	OR	RATE ($)	ADDITIONAL FEE ($)
	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 number found in the appropriate box in column 1.

Legal Instrument Examiner:
/PRASAD JANDHYALA/

This collection of information is required by 37 CFR 1.16. 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 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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============================================================
Sequence Listing was accepted.
See attached Validation Report.
If you need help call the Patent Electronic Business Center at (866)
217-9197 (toll free).
Reviewer: Anne Corrigan
Timestamp: [year=2012; month=7; day=18; hr=8; min=28; sec=46; ms=691; ]
============================================================
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Validated By CRFValidator v 1.0.3

Application No: 13504156 Version No: 1.0

Input Set:

Output Set:

Started: 2012-07-10 11:35:34.797
Finished: 2012-07-10 11:35:38.901
Elapsed: 0 hr(s) 0 min(s) 4 sec(s) 104 ms
Total Warnings: 0
Total Errors: 100
No. of SeqIDs Defined: 100
Actual SeqID Count: 100

Error code	Error Description
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E 257	Invalid sequence data feature in <221> in SEQ ID (2)
E 257	Invalid sequence data feature in <221> in SEQ ID (3)
E 257	Invalid sequence data feature in <221> in SEQ ID (4)
E 257	Invalid sequence data feature in <221> in SEQ ID (5)
E 257	Invalid sequence data feature in <221> in SEQ ID (6)
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Finished: 2012-07-10 11:35:38.901
Elapsed: 0 hr(s) 0 min(s) 4 sec(s) 104 ms
Total Warnings: 0
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No. of SeqIDs Defined: 100
Actual SeqID Count: 100

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

<120> Hybrid Seed Potato Breeding

<130> 05032-00255

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U.S. APPLICATION NUMBER NO.	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
13/504,156	Willem Hendrik Lindhout	05032-00255

22910
BANNER & WITCOFF, LTD.
28 STATE STREET
SUITE 1800
BOSTON, MA 02109-1701

INTERNATIONAL APPLICATION NO.	
PCT/NL10/50716	
I.A. FILING DATE	PRIORITY DATE
10/26/2010	10/26/2009

CONFIRMATION NO. 6337
371 ACCEPTANCE LETTER

OC000000055690527

Date Mailed: 08/01/2012

NOTICE OF ACCEPTANCE OF APPLICATION UNDER 35 U.S.C 371 AND 37 CFR 1.495

The applicant is hereby advised that the United States Patent and Trademark Office in its capacity as a Designated / Elected Office (37 CFR 1.495), has determined that the above identified international application has met the requirements of 35 U.S.C. 371, and is ACCEPTED for national patentability examination in the United States Patent and Trademark Office.

The United States Application Number assigned to the application is shown above and the relevant dates are:

07/10/2012	07/10/2012
DATE OF RECEIPT OF 35 U.S.C. 371(c)(1), (c)(2) and (c)(4) REQUIREMENTS	DATE OF COMPLETION OF ALL 35 U.S.C. 371 REQUIREMENTS

A Filing Receipt (PTO-103X) will be issued for the present application in due course. **THE DATE APPEARING ON THE FILING RECEIPT AS THE " FILING DATE" IS THE DATE ON WHICH THE LAST OF THE 35 U.S.C. 371 (c)(1), (c)(2) and (c)(4) REQUIREMENTS HAS BEEN RECEIVED IN THE OFFICE. THIS DATE IS SHOWN ABOVE.** *The filing date of the above identified application is the international filing date of the international application (Article 11(3) and 35 U.S.C. 363).* Once the Filing Receipt has been received, send all correspondence to the Group Art Unit designated thereon.

The following items have been received:

- Indication of Small Entity Status
- Copy of the International Application filed on 04/26/2012
- Copy of the International Search Report filed on 04/26/2012
- Copy of IPE Report filed on 04/26/2012
- Preliminary Amendments filed on 04/26/2012
- Biochemical Sequence Diskette filed on 07/10/2012
- Oath or Declaration filed on 07/10/2012
- Biochemical Sequence Listing filed on 07/10/2012
- U.S. Basic National Fees filed on 04/26/2012
- Assignee Statement for PGPUB filed on 04/26/2012
- Priority Documents filed on 04/26/2012
- Power of Attorney filed on 07/10/2012

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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
13/504,156	07/10/2012		1545	05032-00255	34	7

CONFIRMATION NO. 6337

22910
BANNER & WITCOFF, LTD.
28 STATE STREET
SUITE 1800
BOSTON, MA 02109-1701

FILING RECEIPT

OC000000055690526

Date Mailed: 08/01/2012

Receipt is acknowledged of this non-provisional 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 APPLICANT, 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 Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections 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**

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Assignment For Published Patent Application

AGVENTURE B.V., Diepenveen, NL

Power of Attorney: The patent practitioners associated with Customer Number 22910

Domestic Priority data as claimed by applicant

This application is a 371 of PCT/NL10/50716 10/26/2010

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

If Required, Foreign Filing License Granted: 07/31/2012

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/504,156**

Projected Publication Date: 11/08/2012

Non-Publication Request: No

Early Publication Request: No
** **SMALL ENTITY** **

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Title

Hybrid Seed Potato Breeding

Preliminary Class

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