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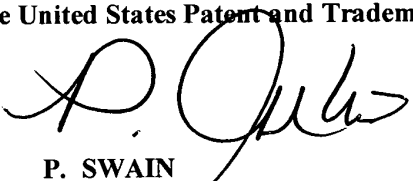
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First Named Inventor/Applicant Name:	Willem Hendrik Lindhout
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1		005032-00197_Application.pdf	1805183	yes	35
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	Specification		1	29	
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2	Provisional Cover Sheet (SB16)	005032-00197_Cover_Sheet.pdf	132188	no	3
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4	Application Data Sheet	005032-00197_ADS.pdf	292969	no	4
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PTO/SB/16 (04-07)

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U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

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Provisional Application for Patent Cover Sheet

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c)

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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):

☒ The address corresponding to Customer Number

☐ Firm or Individual Name

Customer Number

22910

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

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Applicant claims small entity status under 37 CFR 1.27

☐ Yes, applicant qualifies for small entity status under 37 CFR 1.27

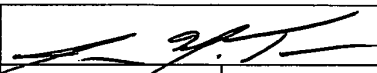
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Signature		Date (YYYY-MM-DD)	2009-10-26
First Name	Jeanne M.	Last Name	Tanner
		Registration Number (If appropriate)	45156

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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
5 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

10

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.

15 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
20 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
25 maximize the yield of harvestable products, compared to 18 kg for maize. In order to break dormancy, seed potatoes should be stored for several weeks before they can be planted. The right conditions during storage such as amount of light, temperature and humidity are crucial to ensure good seed quality.

In addition to the poor seed:harvest ratio, seed potatoes attract and transport pests. These include (amongst others) late blight, Andean potato weevils, nematodes, tuber moths and viruses. The latter are transmitted in the field by aphids and then carried from generation to generation in the seed. Such a virus infection can decrease yields by up to 20 percent. Seed potatoes have high transportation costs because of the great distances between the major seed production areas and the major consumer production areas and the relatively high weight of individual seed potato tubers.

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

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

The arduous task of producing a new potato variety is best understood when understanding the genetics of potato. The commercial potato has a tetraploid genome. Diploid tubers are generally too small for important commercial applications. In addition, the tetraploid genome is extremely heterozygous, often harbouring multiple alleles per locus. It is believed that self-incompatibility, which is primarily experienced at diploid level, and inbreeding depression are responsible for the maintenance of the high genetic variability found in potato and that overdominance of heterozygous alleles (heterosis) results in

vigorous plants. In a typical potato offspring obtained from a cross between two unrelated parental lines deleterious alleles may therefore contribute to either a reduced fitness in case of homozygosity or an increased vigour in case of heterozygosity. It is clear that a breeder needs large populations to maximise the chance of finding individuals that carry a
5 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
10 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
15 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,
20 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
25 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
30 "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
5 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
10 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
15 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.

20 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
25 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
30 heterozygosity than theoretically expected. The slower decline of heterozygosity may be the result of unintentional but unavoidable selection during selfing for the heterozygous plants in the offspring that exhibit higher vigour, fertility and seed germination. It is implied that, as the heterozygosity is reduced by selfing, the fertility and vigour are also

reduced and the plants may become weak and completely sterile. The situation is perhaps worsened by the establishment of homozygous configurations of recessive deleterious genes. This phenomenon which is generally referred to as inbreeding depression has greatly hampered the development for homozygous potato lines and hence the production of uniform hybrid potato seed.

It is an objective of the present the invention to provide means and methods for the production of elite breeding lines of potato and for the production of uniform hybrid potato seed.

10

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:
- 15 (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
 - 20 (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.
 - 25

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.

30

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.

5 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
10 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
15 generations and that may be different from 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
20 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
25 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,
30 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).

10

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.

15 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.

20

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.

30

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
5 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
10 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.

15 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
20 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
25 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.

30 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

5 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-
10 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 an agronomically desirable trait that is
15 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
20 said allele;

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

25 (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
30 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:

- 5 (a) providing a first potato plant, which first potato plant is a plant of a first diploid, self-compatible and essentially homozygous potato line,
- 10 (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;
- 15 (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 and,
- 20 (d) growing said hybrid offspring seed into a hybrid offspring potato plant carrying a chromosome with said allele, and
- 25 (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
30 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
5 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
10 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
15 enhancing the breeding value of the diploid potato breeding line by intercrossing, selfing and backcrossing while continuously 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
20 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
25 the present invention.

DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic presentation of a development scheme for the development of
30 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 Hermesen, 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
5 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:
10 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
15 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

20 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*.
- 25 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
30 that variety or a transgenic derivative having one or more value-added genes incorporated therein (such as herbicide or pest resistance). Backcrossing methods can be used with the present invention to improve or introduce a characteristic into the variety.

The term "crossing" as used herein refers to the fertilization of female plants (or gametes) by male plants (or gametes). The term "gamete" refers to the haploid reproductive cell (egg or sperm) produced in plants by mitosis from a gametophyte and involved in sexual reproduction, during which two gametes of opposite sex fuse to form a diploid zygote. The term generally includes reference to a pollen (including the sperm cell) and an ovule (including the ovum). "Crossing" therefore generally refers to the fertilization of ovules of one individual with pollen from another individual, whereas "selfing" refers to the fertilization of ovules of an individual with pollen from the same individual. Crossing is widely used in plant breeding and results in a mix of genomic information between the two plants crossed one chromosome from the mother and one chromosome from the father. This will result in a new combination of genetically inherited traits. Usually, the progeny of a crossing is designated as: "F1". If the F1 is not uniform (segregates) it is usually designated as "F1 population". "Selfing" of a homozygous plant will usually result in a genetic identical plant since there is no genetic variation. "Selfing" of an F1 will result in an offspring that segregates for all traits that have heterozygotic loci in the F1. Such offspring is designated: "F2" or "F2 population"

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

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

- The term "introgression" refers to the result of an introgression event.
- The term "backcross" refers to the result of a "backcrossing" process wherein the plant resulting from a cross between two parental lines is (repeatedly) crossed with one of its
- 5 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.
- 10 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
- 15 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
- 20 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
- 25 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.
- 30 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
5 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.

10 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
15 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
20 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
25 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,
30 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.

- 5 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.

10

The term "essentially homozygous" refers to a level of homozygosity of at least 50%, preferably at least 75%, 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 more than 10,000 loci.

15

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

- 20 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
- 25 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
- 30 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 $10\% \times 10\% = 1\%$).

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

5

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

10 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.

15

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.

20

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.

30

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.

- 5 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,
10 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.

- 15 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
20 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
25 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,
30 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.

5

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 through 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.

15

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.

20

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).

25

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.

30

The term "self-compatible" refers to capacity of self-pollination, self fertilization and producing fertile progeny.

5 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.

10 ***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
15 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
20 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.

25 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
30 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.

5

NOVEL POTATO PLANTS

Parental breeding lines

10 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
15 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
20 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.

25

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.

30 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;
- 5 - 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
10 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
15 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
20 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
25 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
30 the NCIMB, Aberdeen, Scotland under breeders reference AGVD3, which line represents a BC1 population obtained by crossing a plant of parent P1 as indicated above with a plant of parent P2 as indicated above, and backcrossing the resulting F1 offspring plants with the

P1 parent. It is estimated that AGVD3 is about 75% homozygous for the loci for which the parents differ.

5 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 F₁ hybrid potato seed. Likewise, a
10 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 F₁ seed of a novel potato variety.

The breeding of uniform hybrid seed can be achieved when i) an initial introgression
15 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,
20 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.

25

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
30 essentially homozygous elite potato breeding lines comprising among them a number of agronomically desirable traits selected from the group consisting of insect tolerance, nematode resistance, disease resistance (including but not limited to resistance to Scab caused by *Streptomyces* spp, Powdery scab, *Rhizoctonia*, Silver scurf, *Phytophthora*

infestans), herbicide tolerance, rooting ability, cold tolerance, drought tolerance, wet tolerance, tolerance to dry and wet rot, salinity tolerance, growth rate, tuber development defects (e.g. misshapen or damaged tubers), tuber yield, tuber size, tuber skin color, eye depth, tuber shape, tuber flesh color, tuber taste, tuber storage ability, tuber dormancy
5 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

10

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
15 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
20 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.

25 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
30 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
5 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
10 suitably be introduced from related species (see for instance Hermesen, 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
15 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.

20 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.

25 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
30 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 tetraploid 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.

- 5 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.
- 10 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
15 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
20 (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
25 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
30 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 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
- 5 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.
- 10 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.
- 15 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
- 20 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.
- 25 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
- 30 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.

- 5 Based on the means and methods provided herein, the skilled person will be able to stack new traits into the presently provided self-compatible and essentially homozygous lines in order to establish a population of lines that are enriched for agronomically desirable traits and that can serve as novel germplasm for commercial breeding.

CLAIMS

1. A method for producing a uniform hybrid potato seed, comprising:
 - (a) providing a first potato plant, which first potato plant is a plant of a first diploid, self-compatible and essentially homozygous potato line,
 - (b) providing a second potato plant, wherein said second potato plant is a plant
5 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
10 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,
15 *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,
20 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
25 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
5 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
10 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
15 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,
20 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.
25
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.
- 30 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.

5

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.

10

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

15. A herbicide resistant, nematode resistant, insect resistant and/or disease resistant potato plant produced by the method of claim 14.

20

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 an agronomically desirable trait that is to be expressed in an offspring generation;

25

(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 and,

30

(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
5 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,
10 *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,
15 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
20 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.
25

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.

30 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.

5

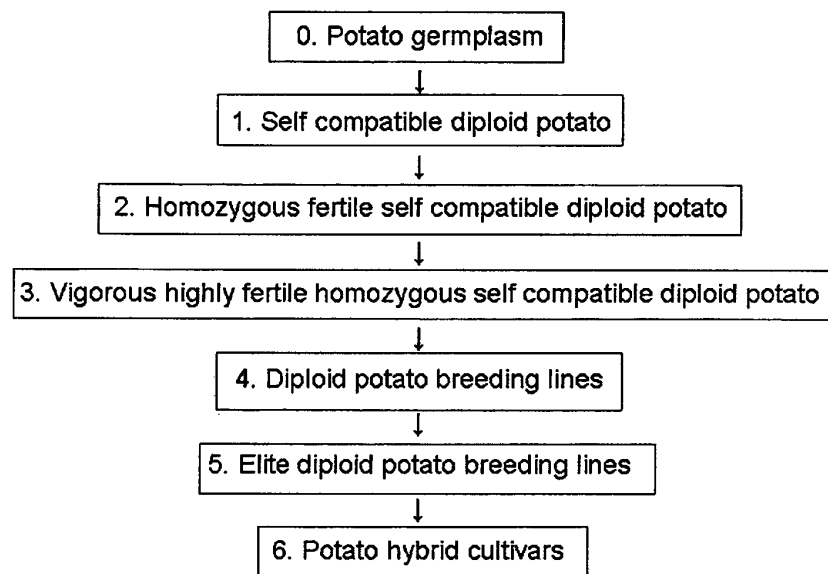
22. A diploid potato breeding line, more preferably an elite line, obtainable by the method of any one of claims 16-21.

23. A hybrid potato cultivar obtainable by crossing a first and second diploid potato
10 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 an agronomically desirable trait that is to be expressed in an offspring generation, and (c) cross pollinating said first and second potato plant such that one of said parent plants produces berries with seeds and collecting said seeds from said berries to provide an offspring generation in the form of a hybrid offspring seed carrying a chromosome with said allele. The invention further relates to seeds thus obtained and to potato plants grown from said seed.

Figure 1



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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. 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:

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		Application Number	
Title of Invention	Hybrid Seed Potato Breeding		
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Citizenship under 37 CFR 1.41(b) i		NL	
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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			

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.

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

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).

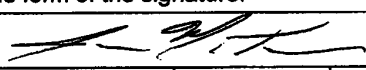
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Application Number	Country ⁱ	Parent Filing Date (YYYY-MM-DD)	Priority Claimed
			☐ Yes ☒ No
Additional Foreign Priority Data may be generated within this form by selecting the Add button.			

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.

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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.**