

P87313US10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Willem Hendrik Lindhout *et al.*,

Serial No.: 13/504,156

Filing or 371 (e) date: 07-10-2012

For: HYBRID SEED POTATO BREEDING

Group Art Unit: 1663

Examiner: Boggs, Russell T

Atty. Dkt. No.: 091876-0014

Confirmation no.: 6337

DECLARATION OF WILLEM HENDRIK LINDHOUT UNDER 37 C.F.R. §1.132.

I, Willem Hendrik Lindhout, hereby declare as follows:

1. I am a citizen of the Netherlands, residing in Wageningen, the Netherlands.
2. I am a named inventor of U.S. patent application No.:13/504,156 to Lindhout *et al.*, assigned to Agventure B.V.
3. I am Founder and Director R&D of Florere B.V., operating under the trade name of Solynta, a privately held Limited Liability Company dedicated to F1 hybrid Potato Breeding and holding company of Agventure B.V.
4. I hold an MSc degree in Biology, an MSc degree in Chemistry, and a PhD in Molecular Biology from the University of Leiden, The Netherlands. Until 2006, I was Associate Professor of Plant Breeding at Wageningen University, The Netherlands. During this period, I published more than 100 scientific publications related to plant breeding, served as the Coordinator of Bachelors and Masters Studies in Plant Sciences at Wageningen University, and served as the National Coordinator of Tomato Genomics Research at the Centre for Biosystems Genomics (CBSG), a national network of biology sciences aiming at a better understanding of the biochemical, molecular and genetic basis of plant species, *c.q.* *Arabidopsis*, tomato and potato. From 2006-2009, I was director R&D of De Ruiter Seeds. After the acquisition of De Ruiter Seeds by Monsanto, I was appointed as Monsanto's Global Breeding Lead of the Vegetable Seed Division. I am currently Research Director at Solynta and Scientific Lead of the National Potato Research Program designated Holland Innovative Potato.
5. I am familiar with the contents of the Office Actions issued by the United States Patent and Trademark Office in respect of U.S. patent application No.:13/504,156, and the claims that are prosecuted thereunder.



6. I understand that the United States Patent and Trademark Office, in assessing the patentability of the referenced patent application, has *inter alia* rejected the claims for failing to comply with 35 U.S.C. §112, i.e. failing to comply with the requirements of written description and enablement and 35 U.S.C. §103 for being obvious in view of the prior art.

Obviousness

7. As I stated during the interview on 30 November 2018 at the USPTO in Alexandria, the generation of homozygous (inbred), diploid potato lines by means of introgressing the *Sli*-gene from a *Sli*-gene donor line into diploid *S. tuberosum* plants followed by inbreeding (selfing) and selection for fertile offspring, which led to the unexpected result of the plants as claimed, was the culmination of at least three years of unsuccessful attempts using four alternative methods. Introgressing the *Sli*-gene into diploid *S. tuberosum* plants followed by inbreeding was the fifth and final attempt that we made in my lab in the years 2006-2008. There was little expectation of success in generating vigorous, fertile plants, as all previous attempts to generate fertile, homozygous, diploid potato lines by introgression of the *Sli*-gene into naturally diploid *Solanum* species such as *S. phureja* had failed.

8. In addition, all commercially relevant *S. tuberosum* plants are tetraploid, and it was believed that diploid *S. tuberosum* would form no useful basis for the production of vigorous commercial potato, since diploids usually have lower yields than tetraploids. In addition, no example was known of a tetraploid vegetatively propagated crop that was converted into a diploid F1 hybrid crop. Tetraploidy was also considered required to overcome the negative effects of the presumed large number of deleterious alleles present in *S. tuberosum*.

9. It was therefore the general belief that a combination of two deleterious alleles of a single gene in a diploid plant (which in a diploid could not be rescued by the additional presence of one or two beneficial alleles as would be the case in a tetraploid plant), would give rise to the phenomenon of inbreeding depression, and that such a phenomenon would be even more severe in *S. tuberosum* due to the fact that such genes can remain "hidden" in the tetraploid genome of *S. tuberosum*. The generation of highly homozygous, diploid lines of *S. tuberosum* was, therefore, *a priori*, not considered feasible due to severe inbreeding depression. All previous attempts to generate vigorous, inbred, diploid *S. tuberosum* potato lines had failed.

10. The claimed step of selecting for fertile offspring during inbreeding of self-compatible *S. tuberosum* was essential to arrive at the method as claimed. As indicated above, tuber yield is the most relevant selection criterion in classical potato breeding. The *Sli*-gene donor material used by the present inventors was obtained from Prof. Hosaka, from the Food Resources Education and Research Center, Kobe University, Japan. Plants are relatively small, with very few flowers/berries, and with extremely low tuber yield, indicative of low vigour and low fertility. The inventors chose to cross the *Sli*-gene donor material with diploid *S. tuberosum*. The hybrid offspring plants were self-compatible, and upon further

selfing, the inventors selected only those self-compatible plants that had seed-carrying berries.

11. This is not an inherent step in potato breeding. Out of the hundreds of offspring plants resulting from a selfing step one could select low fertility plants with high tuber yield for further breeding. As it is undue to maintain each inbred series, selections must ultimately be made in order to keep the program manageable. The present inventors chose to select for fertility. To the surprise of the inventors, a high level of fertility in inbred *S. tuberosum* could be maintained, although tuber yield deteriorated considerably. Yet, against all expectations, successive inbreeding and selection steps resulted in highly homozygous plants with acceptable tuber yields. While fertility could be maintained, yield could even further be improved by intercrossing with other diploid *S. tuberosum* lines followed, again, by inbreeding to generate highly homozygous lines. Eventually, fertile, yet highly homozygous *S. tuberosum* lines were obtained that exhibited remarkably high tuber yields when grown from seed. Two such inbred lines could be crossed to produce F1 hybrid seed, which produced uniform plants. The inventors have presented their findings through journal articles (e.g. Lindhout et al. 2011. Potato Research 54: 301–312), and through presentations at international potato conferences (e.g. EAPR meeting, Oulu, Finland, July 25th, 2011).

12. This notion on the unlikeliness that diploids would provide a useful route to commercial potato production is confirmed in the prior art (Jansky *et al.*, 2009. Advances in Potato Chemistry and Technology Pages 27-62 In: Advances in Potato Chemistry and Technology Chapter 2 - Breeding, Genetics, and Cultivar Development <https://doi.org/10.1016/B978-0-12-374349-7.00002-7>), where it is indicated on page 37, *vide* “2.4.2: Sexual polyploidization”:

“While the development of potato cultivars at the diploid level sounds appealing, it is not likely to be successful. In potato, intralocus (dominance) and interlocus (epistasis) interactions are necessary to maximize yield (Mendoza and Haynes, 1974b).

Tetraploidy offers many more opportunities to create such interactions.”

13. It is also noteworthy that the golden standard in potato breeding is to select suitable breeding material based on tuber yield, as is taught in the prior art (Jansky *et al.*, 2009, *supra*, page 38, first 6 lines of full paragraph):

Initial studies of sexual polyploidization in potato focused on tuber yield and quality. A high degree of heterosis for yield has been demonstrated following unilateral sexual polyploidization in which the tetraploid female parent is typically a potato cultivar or advanced breeding selection and the diploid male parent is a haploid X wild species hybrid or a cultivated diploid X haploid hybrid (De Jong et al., 1981; Bani-Aameur et al., 1991; Tai and De Jong, 1991; Buso et al., 1999a, 2000, 2003; Alberino et al., 2004).

This is understandable, as the single most commercially relevant trait in potato is tuber yield.

14. Our approach differed from that described in the prior art, in that we crossed a *Sli*-gene donor line with diploid *S. tuberosum* plants. This had never been considered or done by anyone else before. Moreover, and perhaps most importantly, we selected for fertility during subsequent inbreeding, thereby completely ignoring the industry standard, tuber yield. We started with three diploid *S. tuberosum* genotypes (D1, D2 and D3, as indicated in Table 6 of the application), which were all successful in that we were able to obtain fertile, highly homozygous (inbred), diploid potato lines. After a number of successive generations, plants started to exhibit considerable tuber yields (up to 300-500 grams), and we continued the breeding program by introgressing genetic material from other genotypes (D4-D16) into the vigorous fertile, inbred, diploid potato lines. This ultimately resulted in plants having tuber yields as high as commercial tetraploid varieties. This was all due to the availability of an inbreeding scheme wherein fertility of the plants was maintained.

Written Description and Enablement

15. I understand that the §112 rejection was, *inter alia*, based on the Examiner's consideration that the inventors were allegedly only successful in producing the inbred, diploid potato lines due to the specific starting material that was used, *i.e.* lines D1-D3 as indicated in Table 6 of the application as filed, and/or the deposited potato lines with NCIMB accession numbers 41663, 41664, 41665, and 41765, as deposited with the NCIMB, Aberdeen, Scotland under breeders reference AGVD1, AGVD2, AGVD3, and AGVD17.

16. Recent whole genotyping by sequencing (GBS) data showed that the genomic variation between diploid potato genotypes, including D1, D2 and D3 is extremely large and represents virtually all variation available in *S. tuberosum*. In fact, the genomic variation in diploid potato germplasm is wider than that within tetraploid *S. tuberosum*. Hence, by showing that the three diploid *S. tuberosum* lines D1-D3 can all successfully be used as starting material, we have shown that there are no limits as to the starting material. More recent, independent research, using completely different diploid *S. tuberosum* clones as recipient of the *Sli*-gene, has confirmed that it is possible to generate fertile, essential homozygous *S. tuberosum* potato lines when selecting for fertile offspring in selfed progenies (Jansky *et al.*, 2014. J Plant Registrations. doi: 10.3198/jpr2013.05.0024crg; Jansky *et al.*, 2016. Crop Sci 56:1412–1422). This independent research supports my statement that the specific starting material is not essential to the success of the claimed method of producing the potato plants, and further supports the effectiveness of the method.

17. The application as filed teaches that selecting for fertile offspring was key to the presently claimed invention. As indicated on page 37, lines 10-13 of the application as filed:

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, page 37, lines 27-32 of the application as filed teach:

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.

18. I therefore affirm herein that we have shown that fertile, vigorous, essential homozygous diploid potato lines can be generated from virtually any self-compatible, diploid *S. tuberosum* plant if one selects for fertile offspring in every generation, and especially in the first five generations of inbreeding.

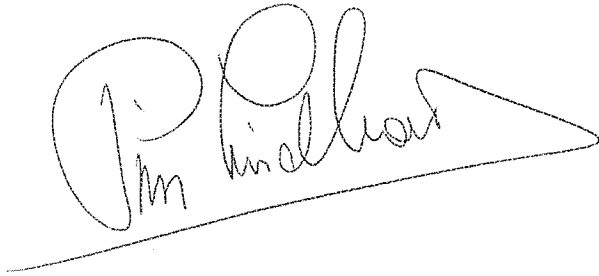
19. A further argument against the Examiner's §112 rejection stating that no structure-function relation was provided for the *Sli*-gene from *S. chacoense*, which was used to render the plants self-compatible. In fact, the *Sli*-gene was already identified by Hanneman in a diploid *S. chacoense* line as early as 1985 (Hanneman, 1985. Am Potato J 62: 428-429). The *Sli*-gene was subsequently mapped to the distal region of chromosome 12 in a cross between *S. chacoense* and *S. phureja* (Hosaka and Hanneman, 1998A. Euphytica 103: 265-271). Thus, prior to the filing date of this patent application, the *Sli*-gene had already been used by several other research laboratories to render naturally diploid wild potato species self-compatible. Thus the *Sli*-gene was already a well-established feature in the prior art.

20. The inventors use the *Sli*-gene as a tool to confer self-compatibility to diploid *S. tuberosum*. To ensure public access to the *Sli*-gene as a tool, and thus enable the claimed invention, the inventors have provided deposits in which a functional and representative form of the *Sli*-gene is present, and which deposits may be used by one of skill in the art to repeat the invention. Alternatively, other sources of the *Sli*-gene can readily be used by one of skill in the art to render diploid *S. tuberosum* plants self-compatible. For instance, a clone of the original material described by Hanneman in 1985 was used as the source of the *Sli*-gene in publications of Jansky (Jansky et al., 2014, *supra*; Jansky et al., 2016, *supra*), and such sources are equally suitable. The presence of the *Sli*-gene can easily be determined by a skilled person by testing for self-compatibility. If needed, one may use markers, but such markers were not used by the present inventors, nor were they used by other researchers who availed themselves of the *Sli*-gene in order to convert self-incompatible potato plants into self-compatible plants (e.g. Hosaka and Hanneman, 1998B. Euphytica 99: 191-197; Birhman and Hosaka, 2000, Genome 43: 495-502; Phumichai *et al.*, 2005, Genome 48: 977-984).

21. I hereby declare that all statements made of my own knowledge are true and all statements made on information are believed to be true and further that the statements were made with the knowledge that willful false statements and the like so made are



punishable by fine or imprisonment or both under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

A handwritten signature in black ink, appearing to read "Dr. Willem Hendrik Lindhout". The signature is written in a cursive style with large, looped letters. A long, thin horizontal line extends from the bottom of the signature to the right.

Dr. Willem Hendrik Lindhout
Wageningen, January 29, 2019