
Hybrid potato breeding for improved varieties

Pim Lindhout, Michiel de Vries, Menno ter Maat, Su Ying, Marcela Viquez-Zamora and Sjaak van Heusden, Solynta, the Netherlands

- 1 Introduction
- 2 The scientific basis for hybrid potato breeding
- 3 The state of the art of hybrid potato breeding
- 4 Production of and commercialization of hybrid seed cultivars
- 5 Inbred lines for genetic research
- 6 Cropping systems based on true seeds
- 7 Case studies
- 8 Conclusion
- 9 Where to look for further information
- 10 Acknowledgements
- 11 References

1 Introduction

The cultivated potato, *Solanum tuberosum*, can be reproduced generatively through seeds and vegetatively through tubers. This may have evolutionary advantages: seeds may provide better survival under extreme conditions, such as frost or drought, and can remain viable in the soil for years. When conditions are mild, tubers survive in a dormant state for a couple of months. When conditions become favourable again, their fast and strong sprouting provides a clear competitive advantage over other plants in the same ecological niche.

In traditional potato breeding, each breeding cycle starts with a cross between two genotypes, usually tetraploid varieties, followed by many years of selection and multiplication (see Chapters 2 and 3). The advantage of this approach is uniformity: the tubers are clones and thus genetically identical. The disadvantage is the low genetic gain in each lengthy breeding cycle, as the genetic composition of the two parental genotypes is just reshuffled, including alleles which negatively affect plant growth and development. As a result, potato yield has not significantly been improved over the past century (Douches

AQ:
Please
check if 'is
less than
a factor
10 per
season' is
OK as is.

et al., 1996; Vos et al., 2015). In addition, the reproduction of seed tubers is less than a factor 10 per season. It takes many years to build up sufficient quantities of seed tubers for commercial production, and the risk of contamination by pathogens increases with each multiplication step.

True potato seed (TPS) has been promoted as an alternative for seed tubers because TPS is easy to store and devoid of most soil-borne pathogens. In South Asia, East Africa and the Andes, TPS is used mainly by subsistent farmers (Almekinders et al., 1996). TPS is produced by crossing parent plants that have been selected to produce a hybrid variety. The parents are propagated vegetatively, similar to seed tuber propagation. As the parents of a TPS variety are heterozygous, all seeds of a TPS cultivar are genetically different. This results in a highly variable crop that is not acceptable in most markets, such as the high value markets of Europe and North America.

Since the success of hybrid breeding in corn in the 1930s, breeders have adapted a hybrid breeding system for many crops (Fig. 1; Crow, 1998; Troyer, 2006; Hua et al., 2003). Typically, hybrid cultivars produce higher yields and show high crop uniformity (Rijk et al., 2013). In addition, the breeding system is fast and efficient and new traits can rapidly be introduced by marker-assisted introgression.

These advantages are also expected for potato: hybrid potato varieties will be higher yielding, will need less crop protection chemicals due to disease resistance and will have better quality for processors and consumers (FAO et al., 2015). A hybrid breeding system for potato offers two additional advantages: fast multiplication of hybrid seeds and easier logistics, as clean true seeds can easily be produced, transported and stored (Duvick et al., 2005).

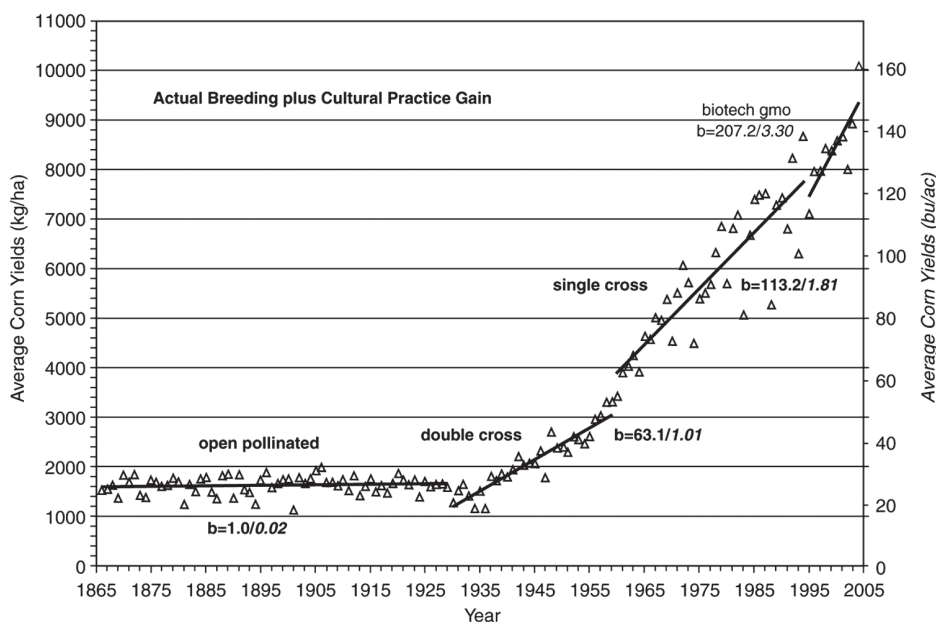


Figure 1 Fivefold increase in corn yields since the introduction of hybrids (Troyer, 2006).

However, self-incompatibility and inbreeding depression have for long hindered progress in hybrid potato breeding (De Jong and Rowe, 1971; Charlesworth and Willis, 2009; Jansky et al., 2016). These two limitations have recently been overcome by introducing a self-compatibility restorer gene and by large-scale and consistent breeding (Lindhout et al., 2011a).

This chapter describes the scientific principles and applied aspects of hybrid potato breeding. The successful introduction of the principle of hybrid potato breeding was described in 2011 (Lindhout et al., 2011a). Since then, we have focused on further developing the potato hybrid breeding system, especially genetic studies to establish a genetics-driven hybrid breeding system. In a recent paper, Jansky et al. (2016) have confirmed the possibilities of such approach.

This is the first publication on the state of the art of a hybrid potato breeding programme. The authors, all working at Solynta, want to emphasize that scientific papers on this topic are not available yet, and hence we have to rely entirely on the results of the Solynta breeding and research programme. Still, in presenting these results, we hope to contribute to a better understanding of the principles and applied aspects of hybrid potato breeding.

2 The scientific basis for hybrid potato breeding

2.1 The principle of hybrid breeding

The basic idea of hybrid breeding is to combine the genes of two parent genotypes, both of which may harbour suboptimal alleles, resulting in weaker performance. If parents have different suboptimal alleles, hybrid offspring can show increased vigour and yield, designated 'heterosis', as the suboptimal alleles in one parent may be compensated by the favourable genes from the other parent (Birchler et al., 2010; Gopal, 2014; Fig. 6). If the parents are completely homozygous, the resulting hybrid offspring will be partially heterozygous and genetically uniform. By testing many hybrid offspring under relevant cultivation conditions, the best combining parents are identified. These are maintained and propagated in separate groups as 'heterotic pools' for further breeding (Brown and Cagliari, 2011).

Thus, hybrid breeding has two distinct processes: development of homozygous parent lines and production and testing of experimental hybrids.

2.2 Diploids are more efficient than tetraploids for hybrid breeding

Homozygous diploids are faster to generate than homozygous tetraploids. For instance, seven generations of selfing are required to obtain 50% homozygous loci starting from a tetragenic tetraploid heterozygote (carrying four different alleles). The same homozygosity level is reached starting from a heterozygous diploid by only one generation of selfing (Haldane, 1930; Fig. 1). For this reason, hybrid potato breeding is more efficient at the diploid level.

2.3 Generation of homozygous diploid potato genotypes via haploidization and via inbreeding

Haploid genotypes can be generated from an egg cell (gynogenesis) or from pollen, often via anther culture (androgenesis). In potato, some haploids have been generated by anther cultures. The resulting haploids were converted into diploids by chromosome doubling. However, the resulting homozygous diploids were very weak and sterile (van Breukelen et al., 1977; Uijtewaal et al., 1987b), hampering their usage in breeding.

Haploidization has been more successful in crossable species like *S. chacoense* (Cappadocia, 1990; Phumichai et al., 2005; Phumichai and Hosaka, 2006) and *S. phureja* (Chani et al., 2000).

A reason for the failure to produce vigorous doubled haploids may be the transition to complete homozygosity in one step. Inbreeding depression may be so severe that homozygous plants are too weak to survive. Repeated selfing, on the other hand, might lead to a more gradual improvement of homozygosity. However, in potato, inbreeding is seriously limited by self-incompatibility, which prevents self-fertilization. Still some rare examples of homozygous diploid plants have been generated by inbreeding but again the homozygous diploid plants always showed a strong inbreeding depression, which limited their usage in breeding (De Jong and Rowe, 1971; Charlesworth and Willis, 2009).

2.4 Large genetic variation in potato causes inbreeding depression

The tetraploid and outcrossing nature of commercial potato is likely responsible for the large genetic variation. In a study on the allelic composition of 800 genes in 83 potato cultivars, an average frequency of 3,2 alleles per locus within a genotype was identified (Uitdewilligen et al., 2013). Among the 83 cultivars, often more than ten alleles per locus

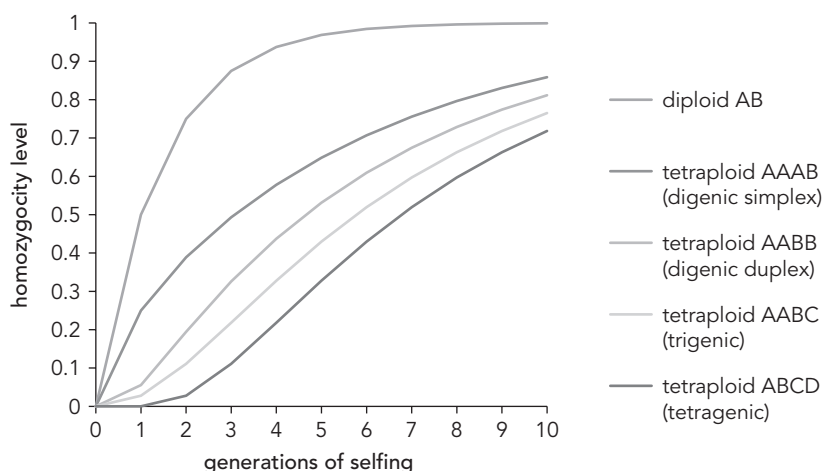


Figure 2 The theoretical increase in homozygosity in diploids and tetraploids through inbreeding, adjusted from Haldane et al. (1930).

were observed. In addition, the frequency of single nucleotide polymorphisms (SNPs) in potato is 1 in 15–30 base pairs (PGCS, 2011; Visser et al., 2014). This means that the genetic distance between the two sets of chromosomes within one diploid potato genotype is four times larger than the genetic distance between the genomes of man and chimpanzee (CSAC, 2005).

The high frequency of allelic variation has the inevitable consequence that ‘weak alleles’ that have a negative effect on plant fitness are maintained. Such alleles remain hidden in the large buffer of four genomes, but reveal themselves upon inbreeding when the chance for homozygosity increases. This is even more manifested at the diploid level where the homozygosity level more rapidly increases upon inbreeding (Fig. 2).

This large genetic variation is also helpful for breeding as it forms a genetic reservoir of useful genes. It is a challenge to identify alleles that contribute most to plant performance. As potato has 39 000 genes, the identification and usage of the ‘best alleles’ per locus, including interactions between them (epistasis), will gradually take place over decades of research and breeding (PGSC, 2011). Corn may serve as a good example, whereby, after a century of dedicated breeding by numerous breeders worldwide, a genetic gain of over 1% per year is still achieved (Troyer, 2006).

2.5 Crossable diploid species and tetraploids increase genetic reservoir for diploid breeding

The potato germplasm available for breeding comprises many species, including diploid species (Jansky and Peloquin, 2006). These have been used as source to introduce resistance genes into cultivated germplasm. Breeders often use diploid potato to rapidly combine favourable traits that can be introduced into the tetraploid germplasm by direct crossings, bridge crossings or via chromosome doubling (De Maine, 1982; Chauvin et al., 2003). The diploid breeding programme at Wageningen University (Hutten, 1994) has generated donor lines that harbour the most important traits for potato breeding (Table 1). Additional diploid germplasm is available from potato gene banks and public research institutes such as University of Wisconsin-Madison, United States; Potato Germplasm Enhancement Laboratory, Japan; Gene Bank at Gatersleben, Germany and International Potato Centre, Peru.

Another source of diploid germplasm is tetraploids that can be prickly pollinated to generate diploid offspring, designated dihaploids (Uijtewaal et al., 1987a). A collection of dihaploids obtained from one tetraploid harbours the full set of genes from the tetraploid and can be exploited in a diploid potato breeding programme.

In conclusion, the large genetic variation in potato and in its wild relatives, combined with the technology to switch between ploidy levels, provides a tremendous wealth of germplasm available for diploid hybrid breeding.

2.6 Diploid potato may perform equal to tetraploids

Most important food crops such as rice, corn and soybean are diploid. Sugar beet cultivars were initially tetraploid, then triploid and since 2000, all new cultivars are diploid. In potato, it has long been assumed that tetraploids outperform diploids (Rowe, 1967; Hutten et al., 1994). Occasional observations have contradicted this assumption: Progeny of diploid potato USW4 with *S. chacoense* M6 produced large tubers and high yield (Lipman and

Table 1 The *Sli*-gene donor, designated DS and 16 diploid potato germplasm, designated D1–D16, used for hybrid breeding at Solynta. The trait abbreviations are according to Hutten (1994)

Abbreviation	Short description
DS	<i>Sli</i> -gene donor
D1	Early (maturity), long, Y, Qcook
D2	Early, Y, Qcook
D3	<i>R3</i> , <i>H1</i> , <i>Gpa2</i> , <i>RXadg</i> , Y (yellow flesh)
D4	<i>Grp1</i> , early, long (shape), <i>Ro1</i> (<i>H1</i> ?)
D5	Early, long, Y, Qfry
D6	Long, Y, Qfry, <i>H1</i> , Qcook
D7	Early, long, Y, <i>H1</i> , Qcook, <i>Zep</i> (orange flesh)
D8	Early, y (white flesh)
D9	Qstarch, Y
D10	Wild species hybrid: <i>phyt avl</i>
D11	Wild species hybrid: <i>phyt rch</i>
D12	Round (shape), Qcook, Qfry
D13	Early, round, <i>Zep</i> , Y, Spectacled, Qcook, blue anthocyan
D14	Wild species hybrid: <i>phyt tar</i>
D15	Wild species BC1: early, <i>phyt vnt1</i> , round, Y, <i>H1</i>
D16	Early, round, y (white flesh)

Zamir, 2007; Jansky et al., 2014). Uijtewaal et al. (1987b) observed that heterozygous diploid potato outperformed all homozygous di- and tetraploid derivatives. These results from potato and from other crops support the expectation that diploid potato will eventually replace tetraploid potato for commercial usage.

3 The state of the art of hybrid potato breeding

In 2008, Solynta started its research by making crosses between diploid potato germplasm, obtained from a pre-breeding programme from Wageningen University (Rutten, 1994), and a homozygous accession of the wild species *S. chacoense*, carrying the dominant self-compatibility controller gene *Sli* (Hosaka and Hanneman, 1998a,b; Phumichai et al., 2005; Lindhout et al., 2011a). The F_1 plants were extremely vigorous and about half of them produced many berries upon self-pollination. This was considered a major breakthrough as these were, to our knowledge, the first vigorous, self-compatible diploid potato plants ever obtained. These F_1 plants were highly heterozygous. The first generation after selfing (designated F_2) should harbour at least 50% homozygous loci. As many of these loci might

harbour 'weak alleles', it was expected that F_2 plants would be too weak to survive. We also made BC_1 populations by backcrossing to the *S. chacoense* parent.

As expected, the F_2 and BC_1 showed weak growth and many died in the field. However, a number of plants survived and 10% of the surviving F_2 plants even proved self-compatible (Lindhout et al., 2011a). We generated the second inbred generation, designated F_3 and made crosses between self-compatible individual F_3 plants. These inbred plants were tested with SNP markers to confirm their genetic identity as real inbreds. These results indicated that breeding hybrid potato was now feasible (Lindhout et al., 2011b).

The weak plant performance, the poor tuber quality and the low yield of the inbreds was not only due to inbreeding depression, but was also caused by the wild *S. chacoense*, a species that hardly produces tubers. So, by this approach, we not only started a hybrid potato breeding system, but also the process of domestication of a new 'diploid potato' based on an interspecific cross of diploid *S. tuberosum* and the wild species *S. chacoense*.

We hypothesized that developing vigorous inbred lines is challenging as for each of the 39 000 loci the most favourable alleles should be identified and combined. Unfavourable genes with large effects on plant performance are identified easily and hence selecting increased plant performance is initially easy and fast. Undesired characters from the *S. chacoense* parent such as abundant stolons, small leaves and twisted, small and low-yielding tubers were removed in a few breeding generations.

Sli is a dominant gene (Phumichai et al., 2005; Phumichai and Hosaka, 2006). However, the successful expression of this gene requires a vigorous plant that is fertile and supports self-pollination. Often these criteria are not met. Therefore, the frequency of self-compatible plants is usually lower than expected based on a monogenic trait. Inbreeding depression is exhibited as weaker plant growth upon higher generations of inbreeding (Fig. 3). As a consequence, the self-compatibility level tends to decrease upon further selfings. The first inbred lines obtained by Solynta, containing over 95% homozygous

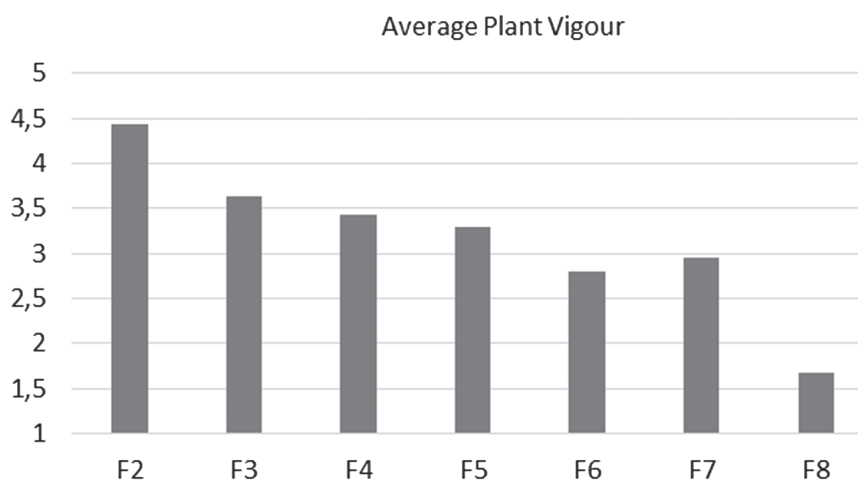


Figure 3 Inbreeding in the diploid Solynta germplasm in winter 2014–15. The F_x indicates $x-1$ generation of selfing after the last cross was made. Data are averaged over the complete trial consisting of over 5000 plants. The scale of plant vigour ranges from 1 = very weak via 3 = average to 5 = very strong.



Figure 4 Examples of Solynta diploid germplasm. Plants were raised from seedlings and grown in the greenhouse in the summer season of 2014 (left panel) and 2015 (middle and right panel). They were among the best genotypes in a breeding programme comprising over 15000 plants.

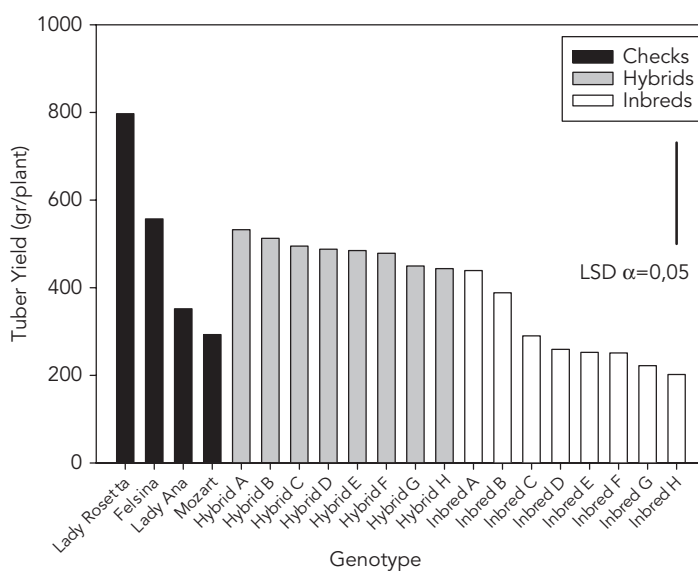


Figure 5 Hybrid performance of the first diploid experimental potato hybrids. Seed tubers were harvested from greenhouse-grown plants, raised in the winter from tubers (checks) and seedlings (hybrids and inbreds). The seed tubers were planted on 8 May 2015 in a trial field on sandy soil in Wageningen and harvested on 17 September 2015.

loci, hardly produced progenies and the seedlings were extremely weak. Therefore, new crosses were made between F_3 and F_5 inbred lines and selfing was started again from these F_1 's to continue selecting parent lines that combine beneficial traits. As a result, performance of the inbreds improved over the following generations (Fig. 4).



Figure 6 Example of heterosis in diploid hybrid potato. The plants were from the same trial as in Figure 5. At the left the female F_3 parent and at the right the male F_5 parent, while the hybrid is in the middle.

In mature hybrid breeding systems, parents of the hybrids are selected based on general combining abilities (GCA), whereby molecular markers can be used to better predict the breeding value of the parents (Tobias et al., 2009). As no historic data were available on combining abilities of diploid potato parents, the selection of the first parents was based on the performance of the parents themselves.

Following the predictions of good performing inbred lines, crosses between these lines were made resulting in 45 potato hybrids which were tested in the field together with 20 inbred lines in two replicates of two plants per plot. The yields varied from 83 to 580 g/plant (Fig. 5). Thirteen hybrids scored higher than any of the inbreds, also outperforming the check variety Lady Anna, while some also showed a strong heterosis for yield (Fig. 6). The trials are being repeated in 2016 by using seed tubers raised from the field.

In addition, a new series of 216 experimental hybrids was generated in the winter-season 2014–15, mainly based on F_6 parent lines. The hybrid seeds were sown immediately after harvest and ten seedlings per hybrid were transplanted into the field in June, 2 months later than the usual seed tuber plantings. Still the yield of some hybrids was higher than 500 g/plant and the tubers were similar in size and shape to commercial seed tubers.

The results of the first experimental hybrids illustrate the potential of diploid hybrid potato varieties. As the first hybrids were randomly made without any a prior information about the combining abilities of the parents, it is expected that the next series of hybrids based on the results of these field trials will perform better and may show a stronger overlap with commercial controls.

4 Production of and commercialization of hybrid seed cultivars

The production of hybrid potato seeds is mainly done by hand pollinations. The seed yield per plant varies from hundreds to many thousands of seeds. Each successful hand pollination generates a berry with 50–150 seeds and each plant produces 5–50 berries. This

is similar to other vegetable *Solanum* crops, like tomato and pepper, where commercial seed is produced by hand pollination.

The emasculation of the flowers is a time-consuming part of the hand pollinations. Male sterility systems have been reported that make emasculations redundant and hence reduce seed production costs (Li, 2008). Alternatively, functional male sterility may be used, whereby pollen is prevented to land on the stigma of the same flower as the exerted stigma may be manually pollinated by pollen of the male parent before the own pollen may reach the stigma (Lössl et al., 2000; Abrol et al., 2012).

The transport of potato seeds over the world is very restricted. This is because the dominant breeding systems are based on the production of seed tubers and hardly any rules are in place for potato seeds. As a consequence, most countries consider potato seeds as belonging to the highest risk classes. This is remarkable as seed tubers may harbour any of over 200 species of pathogens that attack potato (Delleman et al., 2004). In contrast, only six pathogens are seed-borne (Solomon-Blackburn and Barker, 2001). These are five viruses and a viroid, which are absent in major potato-growing regions like the Netherlands. So, potato seeds are very safe and regulations will need to be adjusted accordingly.

The registration process for breeders' rights poses a similar situation: in countries which are members of the International Union for the Protection of new Varieties of Plants (UPOV), the path to commercialization of a hybrid starts with the registration process to obtain breeders' rights, which is based on seed tubers. So, protection by breeders' rights of hybrid potato seed cultivars is not yet possible. The European Union (EU) is adjusting the legislation process, but it may still take several years before this is established. Other non-UPOV countries will likely follow later.

5 Inbred lines for genetic research

Inbred lines allow the generation of mapping populations such as F_2 , BC_1 and BC_2 . As the parents have limited allelic variation, the signal-to-noise ratio is much higher than in studies with heterozygous tetraploid populations. Moreover, putative quantitative trait loci (QTL) can effectively be confirmed in dedicated populations that are selected to segregate for the loci under investigation, and are fixed for other regions on the genome (Wang et al., 2008; Schmalenbach and Pillen, 2009; Fu et al., 2010). In addition, new genetic populations can be generated, which are very powerful for quantitative genetic studies, like nearly isogenic lines (NILs), recombinant inbred lines (RILs) and libraries of introgression lines (Young et al., 1988; Paran et al., 1995; Jeuken and Lindhout, 2004; Finkers et al., 2007; Zhang et al., 2005; Chen et al., 2010; Viquez et al., 2014).

Genetic studies in potato have been done at the tetraploid level and at the diploid level. Tetraploids may support simple genetics like the mapping of resistance genes (Solomon-Blackburn and Barker, 2001), but quantitative studies are less reliable as the genetic noise of the numerous highly heterozygous loci is high (unexplained error). Genome-wide association studies (GWAS) at the tetraploid level will always generate hundreds of potential leads, but only a few hits may be meaningful (Li et al., 2010; D'Hoop et al., 2014). More accurate and reliable quantitative studies were done at the diploid level, initially by crossing heterozygous parents (Prasher et al., 2014) and, more recently, in a diploid F_2 population (Endelman and Jansky, 2016).

The possibility of using self-compatible, homozygous inbred lines for genetic studies offers three powerful advantages:

- 1 Only one allele per homozygous locus is present.
- 2 Only two alleles per heterozygous locus are present.
- 3 Backcrosses and selfings are feasible for confirmation studies.

For more than five decades, mutant studies have uncovered new alleles of important genes and have helped to confirm or determine the function of genes. Although advanced technologies like the CRISPR/Cas system (Belhaj et al., 2013) are likely to replace the methods by which mutants are made, mutants will remain powerful tools to discover unknown phenotypic traits or to study induced alleles that also have the advantage to be free of deregulation rules.

5.1 The first completely homozygous self-compatible diploid inbred line in potato

Most *Solanum* species that are crossable with cultivated potato are self-incompatible. An exception is *S. chacoense* (Hosaka and Hanneman, 1998a; Hawkes, 1990; Jansky et al., 2014) and introducing the *Sli*-gene from *S. chacoense* into cultivated diploid potato resulted in fertile self-compatible offspring (Lindhout et al., 2011a). After several rounds of inbreeding, highly homozygous self-compatible inbreds were generated. The level of homozygosity was assessed by using SNP markers to investigate the effects of inbreeding (Fig. 7). A strong correlation was observed between the overall level of homozygosity and reduced self-compatibility. By new series of crosses, selections and selfings, the agronomic

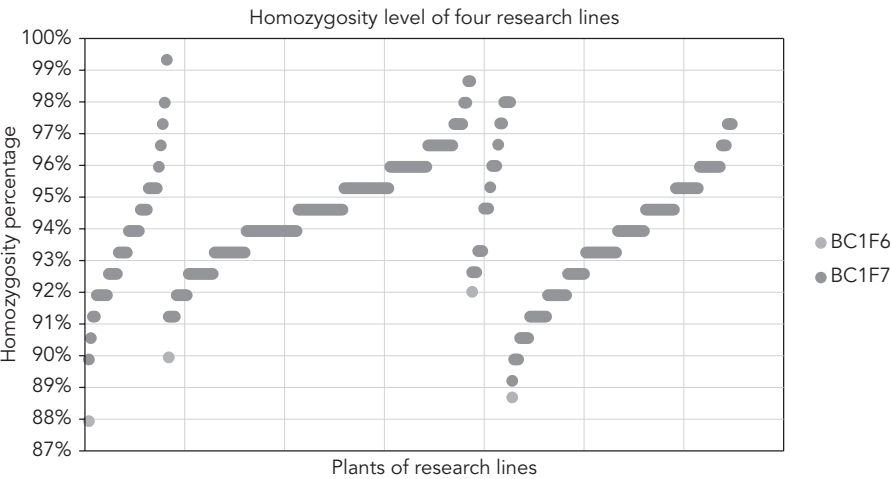


Figure 7 The increase of the level of heterozygosity in the offspring (BC_1F_7) of four BC_1F_6 plants obtained by selfing of the $BC_1(D1 \times F_1[DS \times D1])$ TQ: Please check the usage of brackets is as per style. (Table 1). The width of the bar indicates the number of plants in the progeny within the class with plants of the same homozygosity level. The calculations were based on a total of 150 markers.

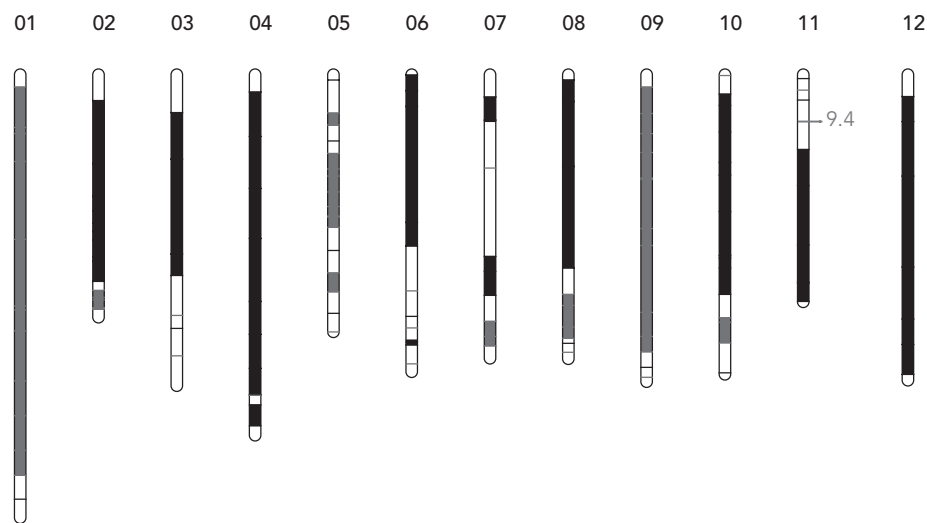


Figure 8 The first essentially homozygous self-compatible diploid potato. The position of the SNP markers is based on the published sequence (PSGC, 2011). The 12 vertical bars indicate the 12 chromosomes. Red bars indicate homozygous D1, blue bars indicate homozygous DS (Table 1). The green line on chromosome 11 indicates a heterozygous scored marker.

performance of the inbred lines continuously improved. After six generations of selfing, a homozygous self-compatible line was generated with only 1 out of 150 SNP markers still heterozygous (Fig. 8). Genotyping by sequencing techniques make it now possible to check the level of homozygosity in more detail.

5.2 Genetic studies in segregating diploid F_2 populations

Recently, Endelman and Jansky (2016) published the first results of a mapping study in an F_2 population of diploid potato. This was based on a cross between the doubled monoploid potato DM1-3 and M6, which is an S_7 inbred line derived from the self-compatible wild relative *S. chacoense*. A single F_1 plant was then self-pollinated and an F_2 population of 109 genotypes was grown, genotyped (>2200 SNPs) and phenotyped. Meijer et al. (2016) analysed an F_2 population (108 markers) based on a cross between two clones, namely DS (a homozygous diploid *S. chacoense* clone containing the self-incompatibility overcoming *Sli*-gene) and D2 (a partly heterozygous diploid *S. tuberosum* clone; see also Table 1). The results of both studies are comparable: tuber shape is associated with a region on chromosome 10, flesh colour with a region on chromosome 3 and tuber and pigment colour on chromosomes 2, 10 and 11. These QTLs were identified at the same loci as described in literature (van Eck et al., 1993, 1994). In both studies, additional QTLs were identified. Furthermore, there is an overwhelming reservoir of potential useful QTLs in the potato germplasm (Bradshaw et al., 2007) and thus also in the dihaploids that can be made. Such QTLs can now be more reliably studied at the diploid level and this will ultimately lead to the identification of the underlying genes. A limited subset of the diploid germplasm may already harbour many important traits for potato breeding (Table 1). Relevant genes

for these traits can be introgressed into vigorous and fertile diploid genotypes to develop progenies with maximum genetic fixation to minimize genetic noise. Such genotypes are unique materials for further detailed genetic studies.

5.3 Marker-assisted backcrossing

Another application for inbred lines in potato is introgression breeding. This requires knowledge of diagnostic markers for the gene of interest, preferably inside the gene, markers for the recurrent parent genome and a self-compatible homozygous backcrossing parent. There are dozens of well-studied resistance genes in potato that can be used for introgression breeding. This paves the way for a marker-assisted backcrossing (MAB) programme in potato (Frisch and Melchinger, 2005).

To this end, homozygous inbred lines are crossed with a diploid donor carrying a specific gene of interest. In two backcrosses, NILs can be generated by selection with diagnostic markers for the gene of interest and against markers in its flanking regions, combined with selection for markers well distributed over the potato genome (whole background selection). Such NILs can harbour over 98% of the recurrent genome in combination with the specific gene. Both parents of a hybrid may have an introgressed gene, resulting in a double stack hybrid. To introgress specific genes in a homozygous parental line will take 2–3 years. These MAB programmes are routinely used in other crops and are also feasible in potato (Mallick et al., 2015; Jeong et al., 2015).

6 Cropping systems based on true seeds

The production of commercial tubers in most parts of the world starts with seed tubers. These have a large reservoir of nutrients for the growing shoots, allow a rapid initial plant growth and fast leaf coverage of the soil, which is one of the most critical factors for potato yield. In contrast, potato seeds are extremely tiny, about 2500 seeds per gram. As a result, during the first period after germination the young seedlings are very vulnerable for abiotic stresses like drought, frost and heat. Field emergence has been reported between 50% and 80% with acceptable tuber yields under different experimental conditions (El-Bedewy et al., 1994; Renia, 1995). However, without a protective environment, the risk is very high that an emerging seedling will not survive, even when it is pelleted or primed.

This chapter describes alternative strategies to circumvent the exposure of week seedlings to harsh conditions.

6.1 Production of seedling tubers in greenhouse

Seedling tubers can be produced under greenhouse conditions by sowing in a medium with sufficient water supply and at optimum germination temperature of 15–20°C (Struik and Wiersema, 2012). As soon as seedlings reach 5–10 cm in length, they are transplanted in pots. The desired tuber size, the available space in the greenhouse and the length of the growing period will determine the pot size, nutrient supply, day/night temperature and light regime (for a detailed protocol see Struik and Wiersema, 2012). Densities of 80–170 plants/m² are common in greenhouses (Lommen, 1995; Tierno et al., 2014). There is a trade-off between number of tubers, size of tubers, planting density and time to harvest.

Depending on the exact conditions, end-product requirements and production costs, the optimal combination is chosen. Alternatively, hydroponic techniques are available, whereby the roots are grown in a dark, humid and soilless environment in two layers, one for nutrient uptake and the other layer for producing mini-tubers (Lommen, 2007). The seedling tubers are picked at regular intervals and the total yield per plant may reach dozens of tubers. The soilless culture assures clean seed tubers. The conditions and picking regime are set to have optimal numbers and tuber sizes.

6.2 Production of seedlings for commercial crop

Greenhouse-grown seedlings can also be used to start the cultivation of a commercial crop. This system is equivalent to the one used for lettuce, leek, cabbage and onion (Leskovar et al., 2014). Technically, commercial potato production from seedlings is feasible and maximizes the benefits of true seeds. When potato transplants are grown as a ware potato crop, a whole new cultivation system must be developed. Important elements are: transplanting systems, plant spacing including ridging or bedding, the use of soil coverage, weed control, irrigation and harvesting methods. Further mechanization and dedicated cultivation systems will be optimized for cropping systems that start with potato seedlings (Roy et al., 2015). In Kenya, tuber yields of 30 tonnes/ha were obtained, whereby seedling transplants were used as starting material for a commercial cultivation (Muthoni et al., 2014). This already represented 50% higher yield than average in Kenya (Wang'om and van Dijk, 2013).

6.3 Seedling tubers as starting materials for a commercial crop

Seedling tubers are equivalent to mini-tubers that are produced from tissue culture, which is routinely done to start a new multiplication round with clean basic seeds (Amin et al., 2014). They are certified as G1 material. The great advantage of seedlings are the reduced costs, compared to *in vitro* grown plants, and the flexibility to start the production whenever and wherever needed, as seeds can easily be stored and transported. As the cost to produce mini-tubers from *in vitro* grown plants is very high, in the present potato system at least three rounds of field multiplications are needed to dilute these high costs over many seed tubers. For a hybrid seed system, the cost of producing seedling tubers is much lower and hence fewer propagation rounds are needed. Therefore, seedling tubers should be multiplied only 1 year and then released to commercial farmers. Such a system also fits better to the fast introduction of new cultivars, which is typical for a hybrid breeding system.

6.4 Production of seed tubers from seedlings in field

At present, the production of seedling tubers is mostly done under tropical conditions. Seeds are sown in a simple greenhouse or in the field under plastic cover with plant densities of 80–100 plants/m² (Kumar, 2014; Struik and Wiersema, 2012; Fig. 9). When the seedlings have reached 5–10 cm in length, they are transplanted to the field, in ridges, at a defined plant density. Additional hilling will increase the number of seed tubers per plant (Wiersema, 1986). To decrease the risk of root damage, the complete substrate is transplanted with the seedling. Plantlets need some time to adapt to outdoor conditions before transplanting directly to the soil (Gopal, 2004). In South Asia, transplants are



Figure 9 The first seedlings of the first diploid hybrid potato hybrids in Democratic Republic of Congo.

planted on the sides of the ridges to benefit from the shade and higher soil humidity. The planting distance may be adjusted to compensate for a shorter growing season compared to seed tubers, if appropriate. The cultivation conditions are similar to traditional potato cultivation systems. In Egypt, seed tuber yields of 40–60 tonnes/ha were obtained in such a system, based on tetraploid TPS populations (El-Yazied et al., 2004).

Seedlings are more sensitive to frost and drought than seed tubers. Thus, transplanting is done in a frost-free season and with irrigation. Compared to the traditional systems of producing seed tubers, whereby the tubers are planted far before the last night with frost, the length of the growing season of transplants may be 2 months shorter. In addition, the plant development may be further delayed due to a transplanting shock and weak initial growth. Hence, tuber numbers are lower and tuber sizes are smaller compared to seed tuber grown plants, causing severe yield reductions. Plant density may be increased to compensate for these reduced yields per plants.

7 Case studies

7.1 Combatting *Phytophthora infestans*

Late blight, caused by the oomycete *Phytophthora infestans*, was responsible for the Irish famine of 1845–47 (Fry, 2008). All potato cultivars were susceptible to the disease and suffered severe yield losses that led to food shortage. Since then, breeders have selected cultivars with field resistance and from the early twentieth century onwards, have introduced specific *R*-genes, often sourced from wild relatives. However, cultivar Pentland Dell, which

carries three *R*-genes, already turned out susceptible to a new virulent race of the fungus even before its widespread use and just 4 years after its introduction (Malcomsum, 1969).

At present, the optimal way to control *P. infestans* is a combined approach of clean seed tubers, clean soils, early and preventive chemical protection and destruction of crop debris after harvest. The global annual cost of cultivation measures and yield loss is estimated at US\$5 billion (Duncan, 1999).

P. infestans reproduces mainly clonally. With hundreds of billions of spores produced per hectare in an infected crop (Skelsey et al., 2010) and a mutation rate of $1:10^9$, mutations in any given gene of *P. infestans* are likely to occur in a disease-infected field. Sexual recombination, combined with the redundancy of several effectors that are recognized by *R*-genes, explains why *P. infestans* easily mutates effector genes and develops virulence (Jiang and Tyler, 2012).

As a result of the high genetic variation in potato, dozens of resistance sources have been identified and are available for breeding (Park et al., 2009). Whereas single genes are easily defeated by virulent races to *P. infestans*, combinations of *R*-genes are more effective, although the Pentland Dell case indicates that a more dynamic approach may be needed (Niks et al., 2011).

Ideally, isogenic cultivars are developed that only differ in the combination of *R*-genes. This would allow to deploy the most suitable cultivar, dependent on the epidemiology of *P. infestans*. However, the introduction of one gene from a wild related species into a tetraploid cultivar by traditional breeding already takes several decades, and to combine different *R*-genes in a breeding programme is simply too complicated.

Since 1990, many *R*-genes to *P. infestans* have been mapped and cloned (Ballvora et al., 2002; Huang et al., 2005; Park et al., 2009). These all belong to the so-called class of 'NBS/LRR genes' and have a cytoplasmic interaction with effector genes of *P. infestans*, resulting in defence responses that block the growth of the pathogen (Jones and Dangl, 2006). A genetic modification (GM) approach to develop a series of isogenic cultivars with different *R*-genes from crossable species is being pursued (Haverkort et al., 2016; Jacobsen and Schouten, 2008). These so-called cisgenic plants might fall under the highly costly and complex GM legislation, which would hamper their commercial opportunities.

The hybrid breeding system offers a clear path towards resilient resistance to *P. infestans*: *R*-genes can be stacked in a potato hybrid via marker-assisted introgression (Park et al., 2009). Two genes can be combined via the two parents in 2–3 years, and additional *R*-genes can be added within a year to generate multi-stack resistance hybrids. In this way, series of *R*-gene isogenic hybrids can be generated as a dynamic resource to select the best combination of *R*-genes to protect the crop against the prevailing races of *P. infestans*.

7.2 Hybrid potato breeding for East Africa

Hybrid potato cultivars will bring great benefits, not only to modern commercial farmers in the developed world, but they may even have a greater social impact in tropical regions where the population rely on potato as a major source of energy and nutrition (FAOstat, 2016). It is very challenging to start an initiative to develop hybrid potato for these regions (Thomas-Sharma et al., 2016; Kumara et al., 2015). When legal and physical protection of the breeding germplasm is not secured in these regions, the development of inbred lines and the hybrid crosses are done elsewhere. The implementation of hybrid potato cultivars in these regions requires considerable investments and strong cooperation of committed

Table 2 Potato area and production in six East-African countries in 2014 (FAOstat, 2016)

Country	Area (000 ha)	Production (000 tonnes)	Yield (tonnes/ha)
Burundi	24.4	181.2	7.4
Kenya	115.6	1626.0	14.1
Rwanda	166.4	2225.1	13.4
Tanzania	211.5	1761.0	8.3
Uganda	39.0	188.0	4.8
Ethiopia	67.4	921.8	13.7

AQ: We have changed 'T' to 'tonnes' in "Table 2". Please check if this is correct?

public and private partners. As an example, the implementation of hybrid potato cultivars for East Africa is briefly described below.

The highlands of East Africa are traditionally important production areas for potato because the crop is an important component of the local diet (Table 2). However, yields remain very low (Table 2). A range of traditional varieties is used from local sources (Kaguongo et al., 2008) as well as improved material from the International Centre for Potato (CIP). Seed tubers are produced by farmers and storage conditions are far from optimal (Kaguongo et al., 2008; Gildemacher et al., 2009). Janssens et al. (2013) concluded that bacterial wilt, lack of clean seed tubers and poor storage are the most prominent production constraints. Gildemacher et al. (2009) showed that only 3% of the seed tubers sold were free of viruses.

True hybrid potato seeds are devoid of contaminating pathogens and therefore offer an excellent opportunity to potato production improvement in East Africa. Such hybrids should be attuned to the needs of the farmers, who grow their crop at the typical local conditions like a short growing cycle of 90–100 days, high temperatures and tuber development under short days. The prerequisites for establishing dedicated hybrid potato breeding system for East Africa are:

- A (private) organization executing a breeding programme tailored to the needs of the region.
- Secured supply of hybrid seeds for the region.
- Regulations supporting imports and exports of seeds, seed tubers and commercial tubers.
- Formal registration system for breeders' rights protection.
- Local organizations testing new experimental hybrids.

Such breeding programme can only become sustainable if the complete downstream part of potato food chain is also well organized. This includes the following:

- Production systems of disease-free seed tubers from seedlings.
- An efficient supply system for farmers to obtain hybrid cultivars.
- Efficient farmers' cropping systems to produce high quality potato tubers for the target markets.
- Efficient logistics to transport farm produce to consumers and processors.

- A well-developed consumer market.
- A business plan over the whole potato chain, whereby all stakeholders benefit.

Solynta is already testing the first experimental hybrids in the highlands of the Albertine Rift in Ituri Province, Democratic Republic of Congo. These are experimental hybrids derived from the European hybrid breeding programme. At a local farm, established by the Lake Albert Foundation, seeds have been sown and seedlings have been transplanted into the field (Fig. 9). These hybrids are evaluated in good cooperation with local farmers and the results are shared with the Solynta breeding programme. These data are used to adjust the selection of inbred lines to the needs in East Africa and to continuously generate new experimental hybrids, which will be tested at the Congo farm again. This iterative and interactive process may already select the first dedicated hybrids for East Africa in 2–3 years.

This breeding programme is accompanied by research on cropping systems for the region. In addition, training programmes for research institutions, agronomists, local staff and interested farmers in the regions will make the farmers' communities and relevant institutions familiar with the new concepts of hybrid potato cultivars. Also market studies are needed to identify and secure stable and sustainable markets for the farmers' potato products. This market may comprise other countries in the Great Lakes Region (South Sudan, Uganda, Burundi and Rwanda).

The support for this programme by national authorities and development agencies – also in the neighbouring countries like Uganda – is required, but it will take time before the concept of hybrid potato cultivars is fully understood and accepted.

8 Conclusion

Since the first crosses in 2008, the Solynta research efforts have been focused on the development of a hybrid potato breeding system. This research has now reached the stage where hybrid potato breeding will become reality. This has recently been supported by two leading potato breeding companies in EU, KWS and HZPC, who have also expressed their conviction that hybrid potato breeding will be future main breeding system (KWS, 2016). In addition, 21 leaders in the industry and potato science in the United States have expressed their opinion on 'Reinventing potato as a diploid inbred line-based crop' with a scientific base for diploid hybrid potato breeding (Jansky et al., 2016).

We have made great advances in the development of useful homozygous inbred lines and the first field evaluations of experimental hybrids have shown the potential of hybrid cultivars to harvest heterosis.

The technologies to develop new cropping systems adapted to various climate zones and agronomic practices, which allow the production of commercial seed tubers from seedlings, are already available.

We envision that future hybrid potato varieties, similar to modern tomato hybrids, will harbour up to 15 resistance genes. In addition, the lifetime of new potato hybrid cultivars will be reduced to less than 5 years as is the experience in sugar beet, where the lifetime of new cultivars is only 2–3 years since the first diploid hybrids have been introduced into the market.

Our inbred lines will also be of great value for research purposes as they allow the development of sophisticated populations that are very helpful in genetic and genomic

studies as has been shown in other crops. This will give a boost in the exploration and exploitation of the genetic variation in the potato germplasm.

The self-compatible potato inbred lines will greatly stimulate quantitative research on the genetic control of complex traits such as interaction with mycorrhiza, biotic stresses, nutrient uptake, nutrition value and processing quality.

The application of hybrid potato breeding technology will not be restricted to the most advanced research groups in the public or private institutions. New players in the scientific and applied breeding field of the potato business will arise and new cooperations will be established to take full advantage of the hybrid breeding technology in science and product development.

Finally, hybrid potato breeding will require the skills of the breeder as well as the knowledge and tools of scientists. As a result, a new generation of potato breeding teams will be established combining the skills of the breeder with the in-depth knowledge of plant scientists.

9 Where to look for further information

This chapter describes the first implementation of a hybrid potato breeding strategy and the progress that is made since 2008. We direct the interested reader to the papers of Almekinders (1996, 2009) to learn about 'conventional TPS'. As a textbook on plant breeding, we suggest Brown and Cagliari (2011), while more advanced potato genetics and genomics is found in Bradeen and Kole (2011). The history and mechanism of *Phytophthora* attack is well described by Fry (2008). Finally, FAO (FAOstat, 2016) statistics provide numerous data on potato cultivation.

Leading centres of research include Wageningen University in The Netherlands, James Hutton Institute in the United Kingdom, the International Potato Centre in Peru, Wisconsin University and Michigan State University both in the United States, whereby the most recent paper of Jansky et al. (2016) can be considered a must for anybody interested in hybrid potato breeding.

10 Acknowledgements

We are grateful to Jan Leemans and Herman Fleer for critically reading and reviewing this manuscript.

11 References

- Abrol, D. P. (2012). Pollination for hybrid seed production. In *Pollination Biology. Biodiversity Conservation and Agricultural Production*. Publisher Springer Netherlands, pp. 397–411.
- Almekinders, C. J. M., Chujoy, E. and Thiele, G. (2009). The use of true potato seed as pro-poor technology: The efforts of an international agricultural research institute to innovating potato production. *Pot. Res.* 52, 275–93.
- Almekinders, C. J. M., Chilver, A. S. and Renia, H. M. (1996). Current status of the TPS technology in the world. *Pot. Res.* 39, 289–303.

- Amin, N., Amin, A. R., Roy, T. S., Ali, M. A., Rashid, M. M., Hossain, M. M. and Hasan, N. (2014). Bulking behavior of seedling tubers derived from true potato seed as affected by its size and harvesting time. *App. Sci. Rep.* 8, 1–8.
- Ballvora, A., Ercolano, M. R., Weiss, J., Meksem, K., Bormann, C. A., Oberhagemann, P., Salamini, F. and Gebhardt, C. (2002). The R1 gene for potato resistance to late blight (*Phytophthora infestans*) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. *Plant J.* 30, 361–71.
- Belhaj, K., Chaparro-Garcia, A., Kamoun, S. and Nekrasov, V. (2013). Plant genome editing made easy: targeted mutagenesis in model and crop plants using the CRISPR/Cas system. *Plant Meth.* 9, 39.
- Birchler, J. A., Yao, H., Chudalayandi, S., Vaiman, D. and Veitia, R. A. (2010). Heterosis. *Plant Cell* 22, 2105–12.
- Bradeen, J. M. and Kole, C. (2011). *Potato Genetics: Genetics, Genomics and Breeding of Potato*. CRC Press, USA
- Bradshaw, J. E., Hackett, C. A., Pande, B., Waugh, R. and Bryan, G. J. (2007). QTL mapping of yield, agronomic and quality traits in tetraploid potato (*Solanum tuberosum* subsp. *tuberosum*). *Theor. Appl. Genet.* 116, 193–211.
- Brown, J. and Caligari, P. (2011). *An Introduction to Plant Breeding*. Wiley-Blackwell, USA, p. 224.
- Cappadocia, M. (1990). Wild Potato (*Solanum chacoense* Bitt.). In Y. P. S. Bajaj (ed.), *Vitro Production of Haploids. Biotechnology in Agriculture and Forestry, Vol. 12 Haploids in Crop Improvement I*, pp. 514–29. Springer-Verlag, Berlin, Heidelberg.
- Chani, E., Veilleux, R. E. and Boluarte-Medina, T. (2000). Improved androgenesis of interspecific potato and efficiency of SSR markers to identify homozygous regenerants. *Plant Cell Tissue Organ Cult.* 60, 101–12.
- Charlesworth, D. and Willis, J. H. (2009). The genetics of inbreeding depression. *Genetics* 10, 783–96.
- Chauvin, J. E., Souchet, C., Dantec, J. P. and Ellisseeche, D. (2003). Chromosome doubling of 2x *Solanum* species by oryzalin: method development and comparison with spontaneous chromosome doubling *in vitro*. *Plant Cell Tissue Organ Cult.* 73, 65–73.
- Chen, X., Niks, R. E., Hedley, P. E., Morris, J., Druka, A., Marcel, T. C., Vels, A. and Wauh, R. (2010). Differential gene expression in nearly isogenic lines with QTL for partial resistance to *Puccinia hordei* in barley. *Genomics* 11, 629.
- Crow, J. F. (1998). 90 Years ago: The beginning of hybrid maize. *Genetics* 148, 923–8.
- CSAC (2005). Initial sequence of the chimpanzee genome and comparison with the human genome. *Nature* 437, 69–87.
- D'hoop, B. B., Keizer, P. L. C., João Paulo, M., Visser, R. G. F., Van Eeuwijk, F. A. and Van Eck, H. J. (2014). Identification of agronomically important QTL in tetraploid potato cultivars using a marker-trait association analysis. *Theor. Appl. Genet.* 127, 731–48.
- De Jong, H. and Rowe, P. R. (1971). Inbreeding in cultivated diploid potatoes. *Pot. Res.* 14, 74–83
- De Mainea, M. J. (1982). An evaluation of the use of dihaploids and unreduced gametes in breeding for quantitative resistance to potato pathogens. *J. Agric. Sci.* 99, 79–83.
- Delleman, J., Mulder, A. and Turkensteen, L. J. (2004). *Potato Diseases: Diseases, Pests and Defects*. Potatoworld and NIVAP, The Hague, the Netherlands.
- Douches, D. S., Maas, D. J., Astrzebski, K. and Chase, R. W. (1996). Assessment of potato breeding progress in the USA over the last century. *Crop Sci.* 36, 1544–52.
- Duncan, J. M. (1999). *Phytophthora*-an abiding threat to our crops. *Microbiol. Today* 26, 114–16.
- Duvick, D. N. (2005). The contribution of breeding to yield advances in maize (*Zea mays* L.). *Adv. Agron.* 86, 83–145.
- El-Bedewy, R., Crissman, C. and Cortbaoui, R. (1994). Progress report. Egypt's seed system based on true potato seed. *CIP Circular* 20, 5–8.
- El-Yazied, A., Elminiawy, S. E., Hamoud, N. K. and El-Kheima, S. (2004). Seed tuber production of some hybrids using true potato seed. Mansoura University. *J. Agric. Sci.* 32, 1329–41.
- Endelman, J. B. and Jansky, S. H. (2016). Genetic mapping with an inbred line-derived F₂ population in potato. *Theor. Appl. Genet.* 1–9

- FAO, IFAD and WFP. (2015). The State of Food Insecurity in the World 2015. Meeting the 2015 international hunger targets: taking stock of uneven progress. Rome, FAO.
- FAOstat. (2016). <http://faostat3.fao.org/home/E>
- Finkers, R., Van Heusden, A. W., Meijer-Dekens, F., Van Kan, J. A. L. and Lindhout, P. (2007). The construction of a *Solanum habrochaites* LYC4 introgression population and the identification of QTLs for resistance to *Botrytis cinerea*. *Theor. Appl. Genet.* 114, 1071–80.
- Frisch, M. and Melchinger, A. E. (2005). Selection theory for marker-assisted backcrossing. *Genetics* 170, 909–17.
- Fry, W. (2008). Plant diseases that changed the world *Phytophthora infestans*: the plant (and *R* gene) destroyer. *Molec. Pl. Path.* 9, 385–402.
- Gildemacher, P. R., Demo, P., Barker, I., Kaguongo, W., Woldegiorgis, G. T., Wagoire, W. W., Wakahiu, M., Leeuwis, C. and Struik, P. C. (2009). A Description of seed potato systems in Kenya, Uganda and Ethiopia. *Am. J. Pot. Res.* 86, 373–82.
- Gopal, J. (2004). True potato seed: Breeding for hardiness. In A. Haneafi (ed.), Sixth Triennial Congress of the African Potato Association. *Proc. APA Congr.*, 5–10 April, Agadir, Morocco, pp. 39–57.
- Gopal, J. (2014). Heterosis breeding in potato. *Agric. Res.* 3, 204–17.
- Haldane, J. (1930). Theoretical genetics of autopolyploids. *J. Genet.* 22, 359–72.
- Haverkort, A. J., Boonekamp, P. M., Hutten, R., Jacobsen, E., Lotz, L. A. P., Kessel, G. J. T., Vossen, J. H. and Visser, R. G. F. (2016). Durable late blight resistance in potato through dynamic varieties obtained by cisgenesis: Scientific and societal advances in the DuRPh project. *Pot. Res.*, 59, 35–66.
- Hawkes, J. G. (1990). *The Potato: Evolution, Biodiversity, and Genetic Resources*. Belhaven Press, London.
- Hosaka, K. and Hanneman, R. E. (1998a). Genetics of self-compatibility in a self-incompatible wild diploid potato species *Solanum chacoense*. 1. Detection of an *S* locus inhibitor (*Sl*) gene. *Euphytica* 99, 191–7.
- Hosaka, K. and Hanneman, R. E. (1998b). Genetics of self-compatibility in a self-incompatible wild diploid potato species *Solanum chacoense*. 2. Localization of an *S* locus inhibitor (*Sl*) gene on the potato genome using DNA markers. *Euphytica* 103, 265–71.
- Hua, J., Xing, Y., Wu, W., Xu, C., Sun, X., Yu, S. and Zhang, Q. (2003). Single-locus heterotic effects and dominance by dominance interactions can adequately explain the genetic basis of heterosis in an elite rice hybrid. *Proc. Natl. Acad. Sci. USA* 100, 2574–9.
- Huang, S., Van der Vossen, E. A., Kuang, H., Vleeshouwers, V. G., Zhang, N., Borm, T. J., Van Eck, H. J., Baker, B., Jacobsen, E. and Visser, R. G. (2005). Comparative genomics enabled the isolation of the *R3a* late blight resistance gene in potato. *Plant J.* 42, 251–61.
- Hutten, R. C. B. (1994). Basic Aspects of Potato Breeding Via the Diploid Level. PhD Thesis, Wageningen University, p. 93.
- Hutten, R. C. B., Schippers, M. G. M., Hermesen, J. G. Th. and Jacobsen, E. (1994). Comparative performance of diploid and tetraploid progenies from 2x.2x crosses in potato. *Euphytica* 81, 187–92.
- Jacobsen, E. and Schouten, H. J. (2008). Cisgenesis, a new tool for traditional plant breeding, should be exempted from the regulation on genetically modified organisms in a step by step approach. *Pot. Res.* 51, 75.
- Jansky, S. H. and Peloquin, S. J. (2006). Advantages of wild diploid *Solanum* species over cultivated diploid relatives in potato breeding programs. *Genet. Res. Crop Evol.* 53, 669–74.
- Jansky, S. H., Charkowski, A. O., Douches, D. S., Gusmini, G., Michael, C., Bethke, P. C., Spooner, D. M., Novy, R. G., De Jong, H., De Jong, W. S., Bamberg, J. B., Thompson, A. L., Bizimungu, B., Holm, D. G., Brown, C. R., Haynes, K. G., Sathuvalli, V. R., Veilleux, R. E., Miller Jr., J. C., Bradeen, J. M. and Jiang, J. M. (2016). Reinventing potato as a diploid inbred line-based crop. *Crop Sci.* 56, 1–11.
- Jansky, S. H., Chung, Y. S. and Kittipadakul, P. (2014). M6: A diploid potato inbred line for use in breeding and genetics research. *J. Plant Registr.* 8, 195–9.

- Janssens, S. R. M., Wiersema, S. G., Goos, H. and Wiersema, W. (2013). The value chain for seed and ware potatoes in Kenya; Opportunities for development LEI. Memorandum 13-080, p. 57.
- Jeong, H.-S., Jang, S., Han, K., Kwon, J.-K. and Kang, B.-C. (2015). Marker-assisted backcross breeding for development of pepper varieties (*Capsicum annuum*) containing capsinoids. *Molec. Breeding* 35, 226.
- Jeuken, M. J. W. and Lindhout, P. (2004). The development of lettuce backcross inbred lines (BILs) for exploitation of the *Lactuca saligna* (wild lettuce) germplasm. *Theor. Appl. Genet.* 109, 394–401.
- Jiang, R. H. Y. and Tyler, B. M. (2012). Mechanisms and evolution of virulence in oomycetes. *Ann. Rev. Phytop.* 50, 295–318.
- Jones, J. D. G. and Dangl, J. L. (2006). The plant immune system. *Nature* 444, 323–9.
- Kaguongo, W., Gildemacher, P., Demo, P., Wagoire, W., Kinyae, P., Andrade, J., Forbes, G., Fuglie, K., and Thiele, G. (2008). Farmer practices and adoption of improved potato varieties in Kenya and Uganda. International Potato Center (CIP), Lima, Peru. Social Sciences Working Paper 2008–5. 85 p.
- Kumar, V. (2014). True potato seed technology – Prospects and problems. In N. K. Pandey, D. K. Singh and R. Kumar (eds), *Current Trends in Quality Potato Production, Processing and Marketing*, pp. 175–82. Central Potato Research Institute, India.
- Kumara, N. S., Govindakrishnan, P. M., Swarooparani, D. N., Nitin, Ch. Surabhi, J. and Aggarwal, P. K. (2015). Assessment of impact of climate change on potato and potential adaptation gains in the Indo-Gangetic Plains of India. *Intern. J. Pl. Prod.* 9 (1), 151–70.
- KWS press release (2016). <http://www.kws.com/aw/KWS/company-info/Products/Potatoes/News-Articles/~hjqx/KWS-to-fully-focus-on-hybrid-potato-bree/>
- Leskovar, I. D., Crosby, M. K., Palma, A. M. and Edelstein, M. (2014). Vegetable crops: Linking production, breeding and marketing. In R. G. Dixon and E. D. Aldous (eds), *Horticulture: Plants for people and places, Volume 1: Production Horticulture*, pp. 75–96. Springer Netherlands, Dordrecht.
- Li, L., Paulo, M.-J., Van Eeuwijk, F., and Gebhardt, C. (2010). Statistical epistasis between candidate gene alleles for complex tuber traits in an association mapping population of tetraploid potato. *Theor. Appl. Genet.* 121, 1303–10.
- Li, X.-Q. (2008). Male sterility systems for hybrid seed production in Brassica crops. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 3, 1–14.
- Lindhout, P., Meijer, D., Schotte, T., Hutten, R. C. B., Visser, R. G. F. and Van Eck, H. J. (2011a). Towards F1 hybrid seed potato breeding. *Pot. Res.* 54, 301–12.
- Lindhout, W. H., Schotte, T. P., Visser, R. G. F., Van Eck, H. J. and Hutten, R. C. B. (2011b). Hybrid seed potato breeding. European Patent Office nr EP 2514303 A1
- Lippman, Z. B. and Zamir, D. (2007). Heterosis: revisiting the magic. *Trends Genet.* 23, 60–6.
- Lommen, W. J. M. (1995). Basic studies on the production and performance of potato minitubers. PhD Thesis, Wageningen Agricultural University, Wageningen, The Netherlands, p. 181.
- Lommen, W. J. M. (2007). The canon of potato science: Hydroponics. *Pot. Res.* 50, 315–18.
- Lössl, A., Götz, M., Braun, A. and Wenzel, G. (2000). Molecular markers for cytoplasm in potato: Male sterility and contribution of different plastid-mitochondrial configurations to starch production. *Euphytica* 116, 221–30.
- Malcolmson, J. F. (1969). Races of *Phytophthora infestans* occurring in Great Britain. *Trans. Br. Mycol. Soc.* 53, 417–23.
- Mallick, N., Vinod, Sharma, J. B., Tomar, R. S., Sivasamy, M. and Prabhu, K. V. (2015). Marker-assisted backcross breeding to combine multiple rust resistance in wheat. *Plant Breed.* 134, 172–7.
- Meijer, D. A., Abdullah, S., Rothengatter, R., Van Eck, H. J., Visser, R. G. F., Lindhout, P. and Van Heusden, S. (2016). An F₂ QTL mapping study in a cross between *S. tuberosum* and *S. chacoense*. *Theor. Appl. Genet.* (submitted).
- Muthoni, J., Shimelis, H., Melis, R. and Kinyua, Z. M. (2014). Response of potato genotypes to bacterial wilt caused by *Ralstonia solanacearum* (Smith) (Yabuuchi et al.) in the tropical highlands. *Am. J. Pot. Res.* 91, 215–32.

- Niks, R. E., Parlevliet, J. E., Lindhout, P. and Bai, Y. (2011). *Breeding Crops with Resistance to Diseases and Pests*. Wageningen Academic Publishers, p. 200.
- Paran, I., Goldman, I., Tanksley, S. D. and Zamir, D. (1995). Recombinant inbred lines for genetic mapping in tomato. *Theor. Appl. Genet.* 90, 542–8.
- Park, T. H., Vleeshouwers, V. G. A. A., Jacobsen, E., Van der Vossen, E. and Visser, R. G. F. (2009). Molecular breeding for resistance to *Phytophthora infestans* (Mont.) de Bary in potato (*Solanum tuberosum* L.): a perspective of cisgenesis. *Plant Breed.* 128, 109–17.
- PGSC (2011). Genome sequence and analysis of the tuber crop potato. *Nature* 475, 189–94.
- Phumichai, C. and Hosaka, K. (2006). Cryptic improvement for fertility by continuous selfing of diploid potatoes using *Sli* gene. *Euphytica* 149, 251–8.
- Phumichai, C., Mori, M., Kobayashi, A., Kamijima, O. and Hosaka, K. (2005). Toward the development of highly homozygous diploid potato lines using the self-compatibility controlling *Sli* gene. *Genome* 48, 977–84.
- Prashar, A., Hornyik, C., Young, V., McLean, K., Kumar Sharma, S., Dale, M. F. B. and Bryan, G. J. (2014). Construction of a dense SNP map of a highly heterozygous diploid potato population and QTL analysis of tuber shape and eye depth. *Theor. Appl. Genet.* 127, 2159–71.
- Renia, H. (1995). True seed is a commercial reality in USA. *Pot. Rev.* 5, 48–51.
- Rijk, B., van Ittersum, M. and Withagen, J. (2013). Genetic progress in Dutch crop yields. *Field Crops Res.* 149, 262–8.
- Rowe, P. R. (1967). Performance and variability of diploid and tetraploid potato families. *Am. Pot. J.* 44, 263–71.
- Roy, T. S., Baque, M. A., Chakraborty, R., Haque, M. N. and Suter, P. (2015). Yield and economic return of seedling tuber derived from True Potato Seed as influenced by tuber size and plant spacing. *Univ. J. Agric. Res.* 3, 23–30.
- Schmalenbach, I. and Pillen, K. (2009). Detection and verification of malting quality QTLs using wild barley introgression lines. *Theor. Appl. Genet.* 118, 1411–27.
- Skelsey, P., Rossing, W. A. H., Kessel, G. J. T. and Van der Werf, W. (2010). Invasion of *Phytophthora infestans* at the landscape level: How do spatial scale and weather modulate the consequences of spatial heterogeneity in host resistance? *Phytopath.* 100, 1146–61.
- Solomon-Blackburn, R. M. and Barker, H. (2001). A review of host major-gene resistance to potato viruses X, Y, A and V in potato: genes, genetics and mapped locations. *Heredity* 86, 8–16.
- Struik, P. C. and Wiersema, S. (2012). *Seed Potato Technology*. Wageningen Academic Publishers, p. 383.
- Su, C. F., Lu, W. G., Zhao, T. J. and Gai, J. Y. (2009). Verification and fine-mapping of QTLs conferring days to flowering in soybean using residual heterozygous lines. *Chin. Sci. Bull.* 6, 499–508.
- Thomas-Sharma, S., Abdurahman, A., Ali, S., Andrade-Piedra, J. L., Bao, S., Charkowski, A. O., Crook, D., Kadian, M., Kromann, P., Struik, P. C., Torrance, L., Garrett, K. A. and Forbes, G. A. (2016). Seed degeneration in potato: the need for an integrated seed health strategy to mitigate the problem in developing countries. *Plant Path.* 65, 3–16.
- Tierno, R., Carrasco, A., Ritter, E. and Ruiz de Galarreta, J. I. (2014). Differential growth response and minituber production of three potato cultivars under aeroponics and greenhouse bed culture. *Amer. J. Pot. Res.* 91, 346–53.
- Tobias, A., Schrag, T. A., Möhring, J., Melchinger, A. E., Kusterer, B., Dhillon, B. S., Piepho, H-P. and Frisch, M. (2009). Prediction of hybrid performance in maize using molecular markers and joint analyses of hybrids and parental inbreds. *Theor. Appl. Genet.* 120, 451–61.
- Troyer, A. F. (2006). Adaptedness and heterosis in corn and mule hybrids. *Crop Sci.* 46, 528–43.
- Uijtewaal, B. A., Huigen, D. J. and Hermesen, J. G. Th. (1987a). Production of potato monohaploids ($2n = x = 12$) through prickle pollination. *Theor. Appl. Genet.* 73, 751–8.
- Uijtewaal, B. A., Jacobsen, E. and Hermesen, J. G. Th. (1987b). Morphology and vigour of monohaploid potato clones, their corresponding homozygous diploids and tetraploids and their heterozygous diploid parent. *Euphytica* 36, 745–53.

- Uitdewilligen, J. G. A. M. L., Wolters, A. M. A., D'hoop, B. B., Borm, T. J. A., Visser, R. G. F. and Van Eck, H. J. (2013). A next-generation sequencing method for genotyping-by-sequencing of highly heterozygous autotetraploid potato. *PLoS ONE* 8(5), e62355.
- Van Breukelen, E. W. M., Ramanna, M. S. and Hermesen, J. G. Th. (1977). Pathenogenetic monohaploids ($2n = 2x = 12$ from *Solanum tuberosum* L. and *S. verrucosum* Schlecht. and the production of homozygous potato diploids. *Euphytica* 26, 263–71.
- Van Eck, H. J., Jacobs, J. M. E., van den Berg, P. M. M. M., Stiekema, W. J. and Jacobsen, E. (1994). The inheritance of anthocyanin pigmentation in potato (*Solanum tuberosum* L.) and mapping of tuber skin colour loci using RFLPs. *Heredity* 73, 410–21.
- Van Eck, H. J., Jacobs, J. M. E., van Dijk, J., Stiekema, W. J. and Jacobsen, E. (1993) Identification and mapping of three flower colour loci of potato (*S. tuberosum* L.) by RFLP analysis. *Theor. Appl. Genet.* 86, 295–300.
- Viquez-Zamora, M., Caro, M., Finkers, R., Tikunov, Y., Bovy, A., Visser, R. G. F., Bai, Y. and Van Heusden, S. (2014). Mapping in the era of sequencing: high density genotyping and its application for mapping TYLCV resistance in *Solanum pimpinellifolium*. *BMC Genomics* 15, 1152.
- Visser, R. G. F., Bachem, C. W. B., Borm, T., de Boer, J., Van Eck, H. J., Finkers, R., Van der Linden, G., Maliepaard, C. A., Uitdewilligen, J. G. A. M. L., Voorrips, R., Vos, P. and Wolters, A. M. A. (2014). Possibilities and challenges of the potato genome sequence. *Pot. Res.* 57, 327–30.
- Vos, P. G., Uitdewilligen, J. G. A. M. L., Voorrips, R. E., Visser, R. G. F. and Van Eck, H. J. (2015). Development and analysis of a 20K SNP array for potato (*Solanum tuberosum*): an insight into the breeding history. *Theor. Appl. Genet.* 128, 2387–401.
- Wang, C. M., Lo, L. C., Feng, F., Zhu, Z. Y. and Yue, G. H. (2008). Identification and verification of QTL associated with growth traits in two genetic backgrounds of Barramundi (*Lates calcarifer*). *Animal Genet.* Vol. 39, 34–9.
- Wang'om, W. G. and van Dijk, M. P. (2013). Low potato yields in Kenya: do conventional input innovations account for the yields disparity? *Agric. Food Sec.* 2, 14.
- Wiersema, S. G. (1986). A method of producing seed tubers from true potato seed. *Pot. Res.* 29, 225–37.
- Young, N. D., Zamir, D., Ganai, M. W. and Tanksley, S. D. (1988). Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the *tm-2a* gene in tomato. *Genetics* 120, 579–85.
- Zhang, Y-M., Mao, Y., Xie, C., Smith, H., Luo, L. and Xu, S (2005). Mapping Quantitative Trait Loci using naturally occurring genetic variance among commercial inbred lines of maize (*Zea mays* L.). *Genetics* 169, 2267–75.