16 Genomics in True Potato Seed (TPS) Technology: Engineering Cloning Through Seeds

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Abstract

Tuber is the main planting material for commercial potato production. Besides, true potato seed (TPS), i.e. true botanical seed is another technology of potato. The TPS-raised crop has several advantages over the tuber-raised crops but due to the certain limitations of the conventional TPS technology, it could not be popularized, as expected the world over. The major drawbacks are non-uniformity of crop and tuber characters, lengthy crop duration, and labour-intensive farming due to seedling raising and transplanting. To address these issues, this chapter highlights a brief overview of the production of hybrid TPS, applying genomics approaches to engineer cloning through apomixis seeds, and offers the prospect of F_1 hybrid potato technology.

16.1 Introduction

Global annual potato crop production has had a massive increase from 160 to 374 million tonnes with a slight increase in its productivity (12.4–19.4 t/ha) during the last six decades. Potato is mainly propagated through tubers. The tuber is the most cost-intensive input which amounts to nearly half of the total production cost. Due to high production costs, the bulky nature of tubers, the logistic problems in storage and transport, and the inadequate availability of quality seed tubers, the replacement rate is very low and therefore the previous season's old tubers are grown, especially in the developing nations. As a result, viral diseases perpetuate in the infected tubers and ultimately result to loss of crop yield.

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Although to produce quality planting materials in a country like India, seed tubers are produced through a seed-plot technique in the plains under a low aphid period zone to minimize virus-vector transmission and minimal viral infections. Besides, such natural conditions are also found in the high hills (>2000 m above mean sea level) where quality seed production is practised. However, due to the low multiplication rate (usually 6–8 times), this is less than can meet the seed demands of the country (Upadhyay 1983). Moreover, in the changing climatic scenario, the window for seed production is likely to shrink and the pressure of pathogens might increase in the near future, which will limit the production of quality planting material. Alternatively, true potato seed (TPS) is a promising technology which has shown potential to produce both disease-free planting material in true seeds form and in a form easy to carry like tomato seeds. Conventional TPS technology has not been proved successful. Therefore, this chapter highlights applications of new genomics tools via apomixis technology and F_1 hybrid technology in potato production.

16.2 Conventional TPS Technology

True potato seed (TPS) is the botanical seed of potato, which is produced by bi-parental sexual crossing. TPS is extracted from a fruit called the ‘berry’ and ~200–250 seeds could be found in a single berry, and 1 g TPS weigh up to ~1500–2000 seeds. In India, there are three hybrid TPS populations: TPS C3 (JT/C-107 × EX/A-680-16), HPS-1/13 (MF-1 × TPS-13), and 92-PT-27 (83-P-47 × D-150), which are recommended for commercial potato cultivation in the country. These are mainly grown in the north-eastern states of the country, where quality seed tubers are not available. Breeding, production and agronomy of TPS are beyond the scope of this chapter, so can be referenced elsewhere (Gaur 1999; Almekinders et al. 2009; Kumar et al. 2009). Figure 16.1 shows the potato flower, fruit (berry) and TPS.

16.2.1 Advantages of Conventional TPS

There are several advantages of TPS technology, as given below:

- TPS is a healthy and disease-free planting material, however, it may transmit potato spindle tuber viroid (PSTVd) and potato virus T.
- TPS is cheaper (nearly 1/10 cost of seed tubers) and affordable for small and marginal farmers.
- It is easy to store for planting, lasting even up to 8–10 years in a refrigerator (4–10 °C) at 3–5% moisture.
- Total cost of potato production using TPS is nearly half that of tubers with equivalent yield.
- Multiplication of quality planting material using TPS is faster.
- Crop production using TPS can be followed by direct seedling, nursery beds and brick-bed tuber-lets methods.

16.2.2 Disadvantages of Conventional TPS

Besides several advantages of the conventional TPS technology, there are several disadvantages of this technology for commercial crop production, as given below.

- TPS-raised crops raised are not uniform in various parameters such as emergence, plant type, tuber traits (shape, size and colour) and crop maturity, and differ compared to tuber-raised crops.
- TPS crop is more labour-intensive especially during seedling raising, transplanting, and further establishment of the commercial crop.
- TPS has a long natural dormancy period of up to one year, and therefore dormancy of fresh seeds is broken with hormonal (GA_3) treatment.

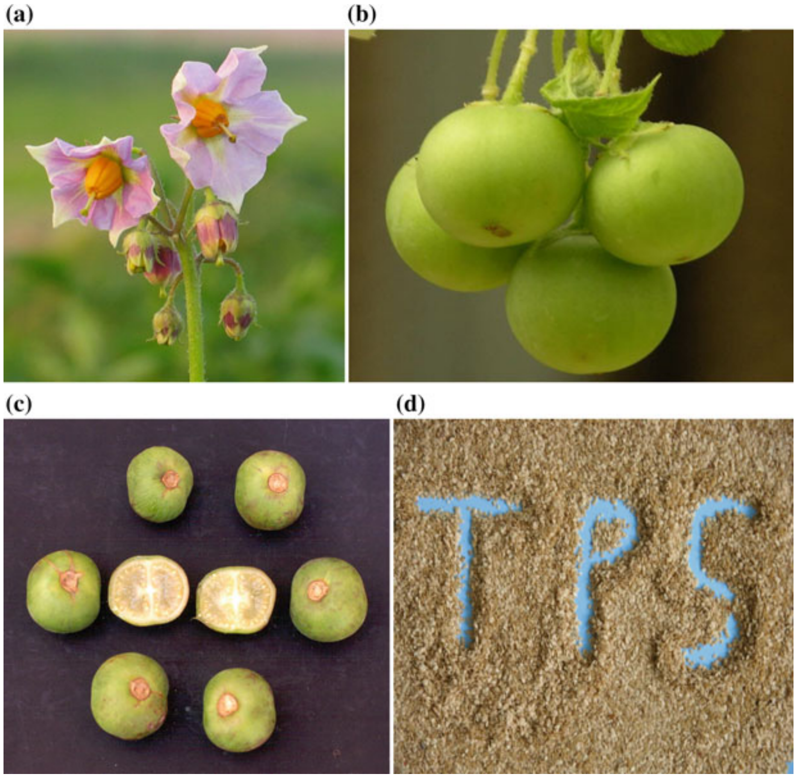

Fig. 16.1 Potato: **a** Flower, **b** fruits (berries), **c** seeds in berries, **d** true potato seeds (TPS)

- A commercial crop raised by TPS takes 15–20 days extra to mature as compared to a tuber-raised crop.

16.3 Prospects of Apomixis in TPS Technology

In flowering plants, two pathways of reproduction exist: (1) sexual, and (2) asexual or apomixis, i.e. asexual reproduction through seeds. The former (sexual) is largely exploited by breeders to breed new varieties though a crossing/hybridization system and also in conventional TPS production, whereas, the latter (apomixis) is continuously receiving increasing attention from both scientific and industrial sectors, as described below.

16.3.1 Synthetic Clonal Reproduction Through Apomixis

Hybrid technology has been exploited from a long time, largely for a tremendous yield increase in seed (sexual) crops like cereals and vegetables. However, hybrid seed has not been used in potato cultivation so far, maybe due to complex genetics and vegetative propagules like tubers. Hence, hybrid technology via synthesis of apomictic TPS could be generated to break the yield barriers in potato, which adds several advantages of TPS. Barring a few exceptions in some forage grasses and fruit trees, apomixis is not a common feature among crops, including potato species. So based on the recent advances in apomixis in *Arabidopsis* applying genome engineering technology, potato holds great promise for the new technology of growing potatoes from botanical seeds instead of tubers. In the coming decades, hybrid potato will play a key role in sustainable agricultural production through the massive multiplication of disease-free and quality potato production to feed billions of people. Thus, the apomixis TPS technology has great potential in the future for the improvement of potato production technology (Barcaccia and Albertini 2013).

Apomixis is a genetically controlled reproductive process by which embryos and seeds develop in the ovule without fertilization. The fixation of a given genotype occurs naturally in species that exhibit an asexual type of seed production termed apomixis. Apomixis produces seed progeny that are exact replicas of the mother plant because embryos are derived from the parthenogenic development of egg cells. Apomixis may be regarded as a consequence of sexual failure rather than as a recipe for clonal success. The major advantage of apomixis over sexual reproduction is the possibility of selecting individuals with desirable gene combinations and to propagate them as clones through seeds. In contrast to clonal propagation through in vitro multiplication, apomixis does not need costly processes such as tissue culture for multiplication. It simplifies the processes of commercial hybrid and cultivar production and enables large-scale seed production economically in both seed and vegetatively propagated crops. In vegetatively propagated crops like potato, the main benefits of apomixis are the avoidance of phyto-sanitary threats and incompatibility barriers (Barcaccia and Albertini 2013).

Apomixis is not a common feature among crop species. Although components of apomixis are found in potatoes, no apomictic potato has been reported so far. Introgression of apomixis from wild relatives into crop species and the transformation of sexual genotypes into apomictically reproducing genotypes are long-held goals of plant breeding. Breeders believe that the introduction of apomixis into agronomically important crops will have revolutionary implications for agriculture. The potential benefits of harnessing apomixis are many and vary from full exploitation of heterosis by reseeding the best hybrids to clonal propagation of the superior genotypes in seed-propagated out-crossing crops. The stabilization of heterozygous genotypes via apomixis would make breeding programmes faster and cheaper. The impact of apomixis in agriculture would be comparable to, or even greater than, the impact of the Green Revolution, especially in Third World countries. Hence, exploitation of apomixis in TPS technology could be an option to break the yield barriers in potato. Apomixis technology could make true potato seeds a more attractive option for potato breeders and cultivators and would return similar benefits to growers.

Harnessing apomixis either through natural or synthetic methods is the major goal in applied plant genetic engineering. Because of its potential in crop improvement and global agricultural production, apomixis is now receiving increasing attention from both scientific and industrial sectors. In this regard, efforts are being focused on genetic studies to analyse apomictic reproduction and to identify the key regulatory genes and mechanisms underlying these processes. Also, investigations on the components of apomixis, i.e. apomeiosis, parthenogenesis, and endosperm development without fertilization, genetic screens for apomictic mutants and transgenic approaches to modify sexual reproduction by using various regulatory genes are receiving major attention. These can open up new avenues for transfer of the apomixis trait to important crop species and will have far-reaching potential in crop improvement. Since natural apomixis is not observed in many of the crop species, and based on the recent advances in de novo synthesis of apomixis in model crop species like *Arabidopsis*, applying genome engineering technology, there is a great promise for application of this novel apomixis technology in potato.

16.3.2 Methods of Apomictic Seed Production

Earlier a few studies have been conducted to demonstrate apomictic seed development in various crops. The recent advance in meiosis (recombination, cell cycle and chromosome distribution) has been reviewed by Crismani et al. (2013), that can be applied to create apomixis and propagate new crop species. Notably, apomictic seeds of *Arabidopsis* were developed by crossing *MiMe* and *GEM* (*dyad/CENH3*) mutants that produce diploid clonal gametes, chromosomes are engineered to be eliminated after fertilization. Up to 34% of the progeny were clones of their parent, demonstrating the conversion of clonal female or male gametes into seeds. Clonal reproduction through seeds can therefore be achieved in a sexual plant by manipulating two to four conserved genes (Marimuthu et al. 2011). Haploid *Arabidopsis* plants were produced through seeds by manipulating the centromere-specific histone protein CENH3. When CENH3 null mutants expressing altered CENH3 proteins are crossed with wild type, the chromosomes from the mutant are eliminated, producing haploid progeny (Ravi and Chan 2010). As CENH3 is universal in eukaryotes, this method may be extended to produce haploids in any plant species. To achieve this, d'Erfurth et al. (2009) created a genotype called *MiMe* (mitosis instead of meiosis) by combining three genes (*SPO11-1, REC8* and *OSD1*) in which meiosis is totally replaced by mitosis. The obtained plants produce functional diploid gametes that are genetically identical to their mother, which is an important step towards understanding and engineering apomixes. Ravi et al. (2008) demonstrated that mutation of the *Arabidopsis* gene DYAD, a regulator of meiotic chromosome organization, leads to apomeiosis, a major component of apomixis. Most fertile ovules in dyad plants form seeds that are triploid and that arise from the fertilization of an unreduced female gamete by a haploid male gamete. The experiments showed that the alteration of a single gene in a sexual crop can bring about functional apomixis.

Engineering cloning through seeds (apomixis) in food crops would help agriculture by fixing hybrid vigour and allow perpetual multiplication of elite heterozygous genotypes. Recent advances in the field of genome engineering are the key to enabling site-directed genome modifications and are sequence-specific nucleases that generate targeted double-stranded DNA breaks in genes of interest, including targeted mutations, gene insertions, and gene replacements. This new technology can be used to knock out the multiple gene of interest rather than introduce foreign genes into plant chromosomes and elucidate gene function and develop new and valuable traits. One of the potential advantages of genome engineering is that one can create a plant that has novel genetic variation and does not have a transgene. Researchers have used meiosis-specific OSD1, SPO11-1 and REC8 genes through genome engineering tools such as ZFNs and TALENs in a model crop plant to develop apomictic seeds. Recently, synthetic clonal reproduction through apomictic seeds has been demonstrated in *Arabidopsis* through genome engineering techniques. Procedures adopted from Marimuthu et al. (2011) are briefly shown in Fig. 16.2. The brief methodology of apomixis (synthetic clonal reproduction through seeds) in *Arabidopsis* (diploid) is described, which can be attempted in potato as well (Fig. 16.3).

16.3.2.1 Step I

The first step in this process is development of *MiMe* (mitosis instead of meiosis) (or *dyad*) mutant for the production of genetically identical diploid gametes.

- During the cell cycle of sexual organism, meiosis reduces the number of chromosomes from diploid to haploid gametes, while, in contrast, mitosis produces two identical daughter cells.
- During meiosis, two rounds of chromosome segregation follow a single round of replication. At meiosis I, homologous chromosomes recombine and are separated, while meiosis II is similar to mitosis (resulting in an equal number of sister chromatids).
- During mitosis, for example, diploid cell chromosomes replicate and sister chromatids segregate to generate daughter cells that are diploid and genetically identical to the initial cell.

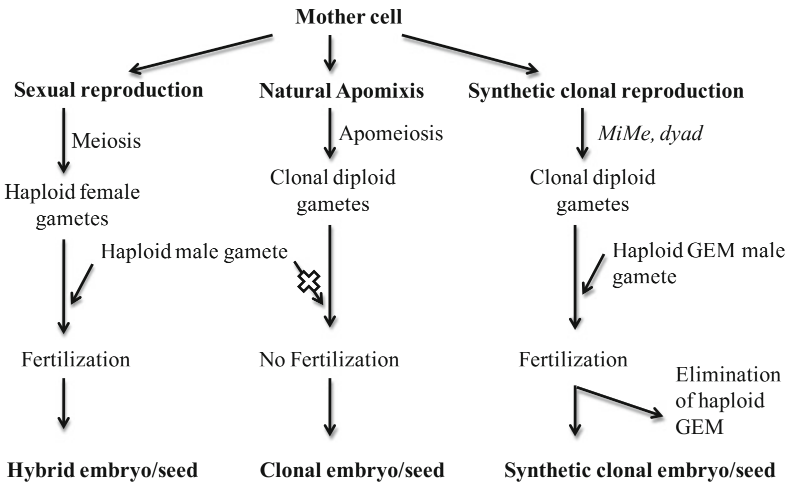

Fig. 16.2 Sexual, natural apomixis and synthetic clonal reproduction systems (main procedures adapted from Marimuthu et al. (2011))

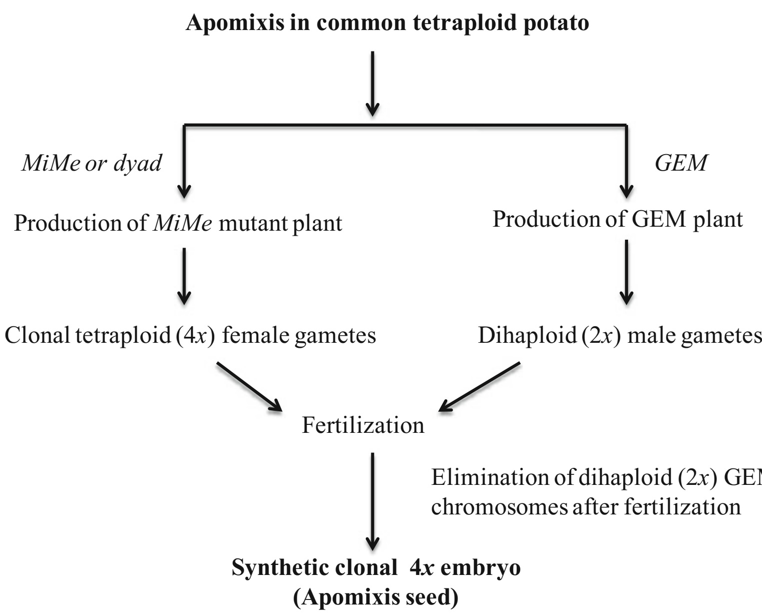

Fig. 16.3 Hypothesis of synthetic clonal reproduction (apomixis) in the common tetraploid potato

- In a sexual plant like *Arabidopsis*, a triple genes mutant called *MiMe* (or *dyad* mutant) is developed by manipulation of three genes in which meiosis is totally replaced by mitosis. In this process, one gene eliminates recombination and the other modifies chromatid segregation.
- The *MiMe* mutant is comprised of three genes, namely, *osd1*, *Atrec8* and *Atspo11-1* in which meiosis is completely replaced by mitosis. A mutant of the two genes *Atspo11-1* and *Atrec8* causes mitosis-like first meiotic division, while the *osd1* mutant prevents a second meiotic division and produces viable diploid male and female gametes. As a result, *MiMe* mutant plant produces diploid (male and female) gametes that are genetically identical to their diploid parent.
- The identical female gametes produced by the *MiMe* (or *dyad*) mutant are used for crossing with male gametes produced from the *GEM* (genome elimination mutant) parent.

However, these *MiMe* clonal gametes participate in normal fertilization but give rise to more chromosome numbers than their parent. Therefore, the *MiMe* clonal gametes would be turned into clonal seeds if fertilized by a genotype whose chromosomes are eliminated after the fertilization from the resultant progenies.

- The replacement of meiosis by mitosis is one of the key components of apomixis, or clonal reproduction through seeds.

16.3.2.2 Step II

The second step in this method is development of a GEM (genome elimination mutant) plant whose chromosomes are eliminated after fertilization.

- Haploids are generally produced by two methods: (1) tissue culture of gametophyte cells, but this may be limited to certain species and genotypes, and (2) haploid are induced from interspecific crosses, in which one parental genome is eliminated after fertilization. In potato, for example, *Solanum phureja* can be used as a male parent for the induction of haploid plant.
- The molecular basis for parental genome elimination is not well understood. However, one theory hypothesizes that centromere from two parents interact unequally with a mitotic spindle in selective chromosome elimination.
- Haploid *Arabidopsis* plants (F_1 paternal or maternal) are produced by crossing the GEM plant and the wild type. The GEM plant is generated through manipulating chromosomes containing the altered centromere-specific histone protein CENH3, whose chromosomes are eliminated after fertilization from the progeny. As CENH3 is universal in eukaryotes, this method may be extended to produce haploids in any plant species. The haploid *Arabidopsis* plants can easily be converted into diploid through somatic chromosome doubling with colchicine treatment.
- The GEM is fertile as male or female, and shows efficient genome elimination when crossed with a parent that produces diploid gametes.

16.3.2.3 Step III

In the process the final step is the production of apomixis seed by fertilization of *Arabidopsis MiMe* or *dyad* mutants with GEM plant.

- The clonal diploid female gametes from *MiMe* mutant are crossed with haploid male gametes from the GEM plant.
- Complete elimination of all the GEM parent chromosomes in the zygote during mitotic divisions.
- Production of clonal embryo identical to mother (female) plant by elimination of GEM male gametes.

Thus, based on the study demonstrated in the model crop *Arabidopsis*, it can be hypothesized that a *MiMe* mutant can be generated from common tetraploid (4*x*) potato for the production of (tetraploid) gametes. Besides, a GEM mutant would produce dihaploid male gamete, and it could also be generated, whose chromosome would be eliminated after fertilization with the *MiMe* parent. Then, a tetraploid apomixis clonal seed could be produced by crossing *MiMe* (female) with the *GEM* (male) parent, as outlined in Fig. 16.3. Nevertheless, this concept has yet to be proven in various crop species including potato.

16.4 F_1 Hybrid Potato

Potato is complicated with its complex genetics, autotetraploid, highly heterozygous, acute inbreeding depression and clonal propagation. Unlike cereals and other vegetable crops, it is evident that hybrid F_1 hybrid technology in potato has not been commercially successful so far. However, theoretically, by using homozygous lines through bi-parental crossing, it is now possible to produce F_1 hybrid seeds. The idea of developing an F_1 hybrid is not new but was proved realistic by Lindhout et al. (2011). Acute inbreeding depression and self-incompatibility of diploid germplasm lines blocked the development of inbred lines. However, back-crossing with lines possessing the *Sli* gene inhibiting gametophytic self-incompatibility produced self-compatible progenies. They demonstrated that fixation of homozygous donor allele is now possible with improvement for tuber traits. To achieve the F_1 hybrid seeds, the development of diploid self-compatible inbred line (M6) is crucial. M6 is a vigorous, fertile (both male and female) line. This homozygous breeding line was derived by selfing of the wild potato species *Solanun chacoense* for many generations, which possess the dominant self-incompatibility inhibitor gene *Sli* and has several desirable traits. M6 can be used to develop recombinant inbred lines for the production of hybrid potato (Jansky et al. 2014). An overview of the procedure of hybrid potato technology is depicted in Fig. 16.4. This elaborates the production of diploid F_1 hybrid potato by crossing homozygous diploid parents and expecting hybrid vigour. Parent 1 (diploid, fertile and self-compatible like M6) is crossed with Parent 2 (diploid parent: D1, D2 and D3) and advance breeding lines were developed for desirable traits by crossing designs (selfing, back-crossing, F3, etc.). Then an F_1 hybrid potato is produced by crossing F_3 materials generated from the cross between Parent 1 × Parent 2. Yield attributes claimed are about 200 g/plant (Patent US 2014/0115736A1). Applying the above concepts, Solynta, a leading potato seed breeding company based in Wageningen in the Netherlands, has developed hybrid potato technology (http://solynta.com/).

16.5 Conclusion

The TPS technology is scientifically sound, technically feasible, economically viable and eco-friendly. TPS provides an opportunity even to small farmers to generate high quality planting material and assures high yields with low inputs as compared to the tuber seed of unknown health status. Although there are some bottlenecks that hinder the adoption of TPS among farmers, those could be overcome by further improvement in parental lines by the introgression of desired traits from diverse genetic resources and adjusting the planting dates, as well as agronomic practices according to the local conditions. The TPS systems have encountered major problems such as poor germination rates, non-uniform tubers, long dormancy and increased irrigation needs. Finally, TPS technology remains to be seen as a ready-to-use technology with the economic comparison of TPS versus seed tubers in the dynamics of cropping system for potato production.

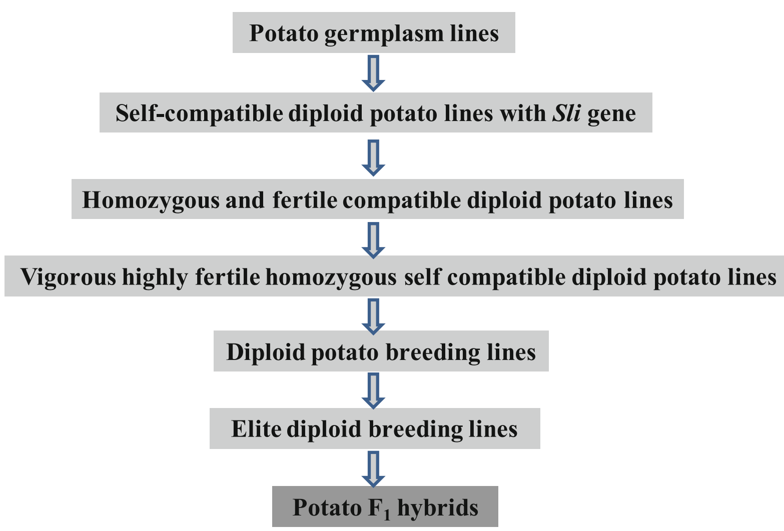

Fig. 16.4 Procedure of F_1 hybrid potato seed production using self-compatible diploid inbred lines (original procedures adapted from Patent Application Publication: US 2014/0115736A1)

In the era of modern genomics tools, production of apomictic seed holds great promise for growing potato from botanical seeds instead of tubers. It is explained that apomixis results from the combination of abnormal meiosis, abnormal fertilization and parthenogenesis. The apomictic seed production technology could make TPS a more attractive option for potato breeders and growers and such an approach would assist the expansion of potato acreage in the tropics. Although novel genomics tools have been tested so far only in the model plant *Arabidopsis*, this concept of apomictic seed development in potato remains elusive. Engineering cloning through apomictic TPS in potato would revolutionize agriculture by fixing hybrid vigour and allowing the perpetuation of elite heterozygous genotypes. To develop an F_1 hybrid potato, the fixation of homozygous donor alleles has been demonstrated using *Sli* (S-locus inhibitor) gene donor parents that gives a self-compatible potato in the diploid breeding programmes. In addition, an F_1 hybrid potato would be likely to become more important and increase potato research and development.

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