

Apomixis: new horizons in plant breeding

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Abstract: Apomixis is seed formation without fertilization, commonly observed in wild plant species. It has the advantages of clonal reproduction and propagation by seeds. Although it has a complex inheritance, genes controlling apomictic reproduction are being identified. Transfer of apomixis to crop species through wide crosses has not been successful so far, but transgenic technology offers a more powerful way to introgress apomixis into crop species. When crop species acquire this trait, superior genotypes, including hybrids, will be fixed and seeds will be multiplied without segregation of superior gene combinations. In addition, it will make hybrid production possible in all crops, lower the costs of hybrid seed, allow farmers to produce their own seed, eliminate crop losses due to pollination failures, and shorten the time to improve new varieties since the breeding will generally be completed by a single cross. In clonally propagated crops, apomixis will eliminate expensive and difficult tissue culture procedures needed for rapid and disease-free multiplication, make storage of planting material easy, and allow the crop to be saved and used as planting material (e.g., potato tubers). Although there are some concerns about biological safety of apomictic crops, they can be easily resolved by refining the apomixis technology. Therefore, it seems that apomixis is about to change the face of plant breeding forever.

Key words: Apospory, diplospory, clonal propagation, conditional apomixis, hybrid seeds, plant breeding

1. Introduction

Apomixis is a mechanism of seed formation without fertilization and is observed in more than 300 species in 30 out of 460 angiosperm families, but it is not common in crop species (Bashaw, 1980). It was considered as an obstacle for plant breeding. Indeed, the presence of apomixis in the species *Hieracium*, in which Mendel was asked to prove the genetic principles he had developed in peas, caused him to withdraw from the scientific world due to his failure to do so (Carneiro et al., 2006). In recent years, however, apomixis is seen as a way to maintain superior genotypes as clones of seeds. As such, apomixis has been a major area of investigation in plant genetics.

Unlike in animals where it is a hot topic nowadays, cloning is an ordinary process in plants. Many plant species can be propagated using various plant parts. Clonal propagation has some advantages as well as disadvantages. Apomixis combines the advantages of propagation by seed (higher multiplication rate, easier storage and planting, suitability for machine planting, less seed material use, and less bearing of diseases) with those of propagation by clone (maintaining genetic structure and hence fixing superior genotypes after crossing). However, apomixis manifests itself in different mechanisms that have not yet been fully elucidated. Genetic studies reveal that,

though complicated, the trait is controlled by a few genes (Barcaccia and Albertini, 2013). In addition, different phases of apomictic reproduction have been achieved artificially (d'Erfurth et al., 2009). Apomictic reproduction of crop species would lead to revolutionary developments in terms of crop improvement. The benefits of apomixis are estimated to surpass those of the green revolution (Grossniklaus et al., 1998).

The aim of this review paper is to discuss the use of apomixis in plant breeding. Our main attention is on its benefits in crop improvement rather than its physiological nature or molecular mechanisms. Methods to achieve apomixis in crop species and its economic advantages are also discussed.

2. Mechanisms of apomixis

There are various mechanisms of apomixis, some of which result in unstable chromosome numbers (Naumova, 1993). Here we only discuss the ones with stable chromosome numbers and those that are important for plant breeding purposes without going into much detail.

Development of apomictic seed formation takes place in three stages: a) suppression of meiosis (apomeiosis), b) endosperm formation without fertilization (parthenogenesis), and c) seed formation with (pseudo-

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apomixis) or without (autonomous apomixis) fertilization. In its simplest form (adventitious embryony), apomixis takes place without formation of an embryo sac. In other words, the process begins directly from a somatic cell in the nucellus or inner integuments, bypassing meiosis altogether (also called sporophytic apomixis). These cells differentiate and form an embryo. In species with endosperm development, the endosperm is formed by polar nuclei. Adventitious embryony is common in *Citrus* species, mango, and orchids.

Apomixis in which the embryo develops from a diploid egg in the embryo sac is called gametophytic apomixis and has two types, apospory and diplospory. In apospory, the embryo and endosperm develop in unreduced embryo sacs in the ovule. In aposporous apomicts, the megasporangium cell in the sexual ovule starts to develop but stops at some stage. One or more somatic cells in the ovule and their nuclei start to develop and they generally resemble megasporangium mother cells. At a stage before the mature embryo sac formation, the megasporangium or young embryo is aborted and replaced by developing aposporous sacs. Apospory is by far the most common mechanism in higher plants (Bashaw, 1980) and has been reported in *Beta*, *Brachiaria*, *Cenchrus*, *Chloris*, *Compositae*, *Eriochloa*, *Heteropogon*, *Hieracium*, *Hyparrhenia*, *Hypericum*, *Panicum*, *Paspalum*, *Pennisetum*, *Poaceae*, *Ranunculus*, *Sorghum*, *Themeda*, and *Urochloa* (Barcaccia and Albertini, 2013).

In diplospory, the embryo and endosperm develop in an unreduced embryo sac derived from the megasporangium mother cell, which differentiates as in sexual ovules but does not undergo meiosis. Diplospory is found in *Tripsacum*, *Eragrostis*, and *Taraxacum*. Apomictic female gametes (2n) undergo embryogenesis autonomously. Apomictic plants carry functional pollen, which they sometimes need for endosperm formation.

Observation of apomixis is difficult since it is generally accompanied by sexual reproduction, also called facultative apomixis. Sexual reproduction percentage varies by environmental conditions. A predominance of maternal type plants indicates apomixis. For a conclusive evaluation, controlled crosses and, ultimately, histological examination in which embryo sac development is monitored are necessary.

3. Genetic basis of apomixis

Although apomixis is a qualitative trait controlled by a few genes (Hanna et al., 1998), it involves many intriguing aspects of genetic analysis. First of all, since it is not common in cultivated species, genetic studies of apomixis have been conducted in relatively unknown polyploid and heterozygous wild plants. There is a striking association between gametophytic apomixis and polyploidy (Savidan, 2000), which might involve dosage effects of genes (Quarin, 2001). However, discovery of apomixis in diploid populations indicates that polyploid genome structure is not an absolute necessity for apomixis (Rodriguez-Leal and Vielle-Calzada, 2012). In addition, there are epistatic interactions between genes controlling apomixis. Extraordinary conditions such as sporophytic and gametophytic factors, modifiers

of gene expression, segregation distortion, suppressed recombination, environmental effects, and presence of asymmetric genome regions are all involved in shaping the apomictic phenotype. Low pollen fertility, interspecific crosses, genome irregularities, difficulties in observing apomixis, and the occurrence of both apomictic and sexual reproduction in the same plant add to the difficulty of genetic studies of apomixis. Furthermore, evidence of the epigenetic control of apomixis has come from recent studies (Ortiz et al., 2013). All these factors probably mean that apomixis is conditioned by different and alternative mechanisms in different plant species.

Most studies about the inheritance of apomixis point to a single gene with some modifiers. There is a consensus that both apospory and diplospory in grasses are controlled by a dominant locus (Carneiro et al., 2006). Similarly, studies in *Panicum maximum* (Savidan, 1981) and *Hieracium aurantiacum* (Bicknell et al., 2000) indicated a simple dominant factor for apomixis. Early studies showed a tight association between suppressed meiosis (apomeiosis) and seed development without fertilization (parthenogenesis), and it was thought that these two traits were pleiotropic. However, later studies with the genera *Allium*, *Poa*, *Erigeron*, *Taraxacum*, *Hieracium*, *Hypericum*, and *Potentilla* revealed the independent control of apomeiosis and parthenogenesis (Ozias-Akins and van Dijk, 2007).

Studies using DNA markers showed that recombination is seriously suppressed in a 25–30 cM chromosomal region associated with apomeiotic control in some genera such as *Pennisetum* and *Tripsacum* (Grimanelli et al., 1998). Furthermore, this region was found to have disomic inheritance in polyploid species (Stein et al., 2004). This recombination-suppressed region is not conserved among different grass species. A genomic region was detected in apomictic *Pennisetum* plants whose homolog was missing in sexual relatives. Gene(s) controlling apomixis also have been associated with some lethal factors affecting female or male gametes (Grimanelli et al., 1998).

Mutation studies give significant clues about the genetic mechanism of apomixis. Deletion studies in a *Hieracium* species of the family Asteraceae showed that apospory is constituted by two dominant genes named *LOA* and *LOP* (Koltunow et al., 2011). *LOA* conditions aposporous cell differentiation and suppression of sexually developing megasporangia, while *LOP* mediates autonomous development of the embryo and endosperm. Mutations in *LOA* and *LOP* result in sexual reproduction. Accordingly, apomixis is conditioned via the breaking down of the default sexual pathway by two different loci.

4. Conversion of sexual crops into apomictic ones

There are various ways of converting crop plants into apomictic ones: a) wide crosses with apomictic wild relatives, b) mutation, and c) genetic transformation.

Transfer of apomixis from wild relatives via sexual hybridization depends on the presence of relatives with which interspecific hybridizations can be made. For most cultivated species this is not possible. Apomixis was transferred into maize from its relative *Tripsacum* through hybridization. Since the hybrids obtained after a series of

backcrosses were completely sterile, facultative apomicts were needed for recovery of the maize genome. The absence of such plants made introgression of the character difficult. Although significant developments have been made recently, it is generally accepted that apomixis transfer via wide crosses has been unsuccessful so far (Spillane et al., 2004).

Studies in the model plant *Arabidopsis* revealed that apomixis can also be achieved through artificial mutations in addition to the natural mechanisms observed in plant kingdom. Apomeiosis, the first stage of apomictic reproduction, has been artificially obtained using at least two mechanisms so far. First, mutation in a gene named *Osd1*, preserved throughout the plant kingdom, eliminates the second division in meiosis (d'Erfurth et al., 2009). When the *Osd1* mutation was combined with two other mutations that eliminate recombination and modify chromatid segregations, a genotype called MiMe was obtained in which meiosis was replaced with mitosis. MiMe plants produce diploid male and female gametes with the same genetic composition as the mother plant, but the chromosome number is doubled in each generation. In the second mechanism, a mutation in the *Arabidopsis SWI1* gene leads to apomeiosis and diploid egg formation (Ravi et al., 2008). Fertilization of these eggs with reduced normal pollen produces triploid seeds. Both of these mechanisms need a way of eliminating the paternal chromosome since the seeds they produce have higher ploidy levels than that of the species. Such elimination was conditioned by modifications in a single centromere specific protein, CENH3 of *Arabidopsis* (Ravi and Chan, 2010). In this system, which also provides a way of producing haploid plants, when the CENH3 mutant was crossed to wild-type plants, chromosomes of the mutant plant disappeared and only the chromosomes of the wild-type plant remained. Although all of the plants produced were not diploid and fertilization was needed for seed development, results from *Arabidopsis* MiMe plants prove the possibility of artificially bringing apomixis into a new species. The second stage of apomixis was also achieved by mutations. Chaudhury et al. (1997) reported seed development in mutations of *Arabidopsis FIS1*, *FIS2*, and *FIS3* genes in the absence of fertilization. This finding indicates that, if the effects of genes rendering normal seed development that are epistatic to apomixis genes are eliminated, genes controlling the stages of apomictic reproduction could become active.

Transfer of apomixis through genetic transformation into crop plants is a potent approach but is not feasible at the moment since the genes controlling apomictic reproduction have not yet been cloned. Map-based cloning of involved genes is slow due to suppressed recombination and abundance of repeated DNA sequences in genomic regions associated with apomixis. In some apomictic species, apomixis genes were found using deletion studies. Cloning of the abovementioned *LOA* and *LOP* genes of *Hieracium* is underway and, after their cloning and transfer, apomixis could be introgressed into other crop species (Kotani et al., 2014).

In order to fully benefit from apomixis, apomictic crops should have certain characteristics. First of all, they should retain their ability to sexually reproduce. Second, they should have blocked male organ development to save precious resources and to prevent horizontal transfer of apomixis genes into wild relatives. Finally, these crops should have autonomous endosperm development to prevent crop losses due to pollination failure. As shown in maize, a 2:1 maternal-to-paternal genome ratio is critical in endosperm development in most species (Baroux et al., 2002). In artificial apomicts, this ratio is 4:1, which prevents endosperm development. In apomictic *Paspalum notatum*, on the other hand, endosperm development is independent of genome ratios (Quarin, 1999). *Paspalum*-type autonomous endosperm development is critical in crop species to prevent seed yield losses.

Recently, a "conditional apomixis" approach in which apomictic reproduction is temporarily switched to sexual reproduction (or vice versa) has gained importance. Here, apomictic reproduction could be the default pathway and sexual reproduction is temporarily activated, or sexual reproduction is the default pathway and apomixis is activated only for seed multiplication (Spillane et al., 2004). The latter is preferred for environmental considerations (Spillane et al., 2004), but it will prevent farmers from producing their own seeds. Conditional apomixis can be achieved using special promoters whose expression level is changed by certain chemicals (Spillane et al., 2004) or epigenetic factors (Pupilli and Barcaccia, 2012).

5. Advantages of apomixis in plant breeding

Apomixis is basically a way of multiplying superior genotypes, including hybrids, as clones, but in the form of seeds. Hybrid varieties are common in cross-pollinating crops because of their superior yield performance. Due to the complexity and time required by hybrid variety development programs and the fact that hybrids cannot be multiplied simply by growing them, seeds of these varieties are extremely expensive (Figure 1). On the other hand, it is possible to multiply apomictic hybrid seeds forever since they are clones. This will cause enormous reductions in the price of apomictic hybrid seeds. Another factor lowering the cost of apomictic hybrids is that the farmers could produce their own seeds, which is not possible in classical one-way hybrids because of inbreeding effect and decreased performance. Since the genetic composition of an apomictic *F₁* hybrid is maintained, farmers do not have to buy seed each year, but should buy certified seed every 5 years as in most pure-line cultivars.

Apomictic hybrids will not need cytoplasmic male sterility and fertility restorer systems, which means much shorter and easier hybrid development procedures (Figure 1). Besides increasing the time needed to develop hybrid varieties, these systems also cause problems by making hybrid varieties dependent on a limited number of sterility

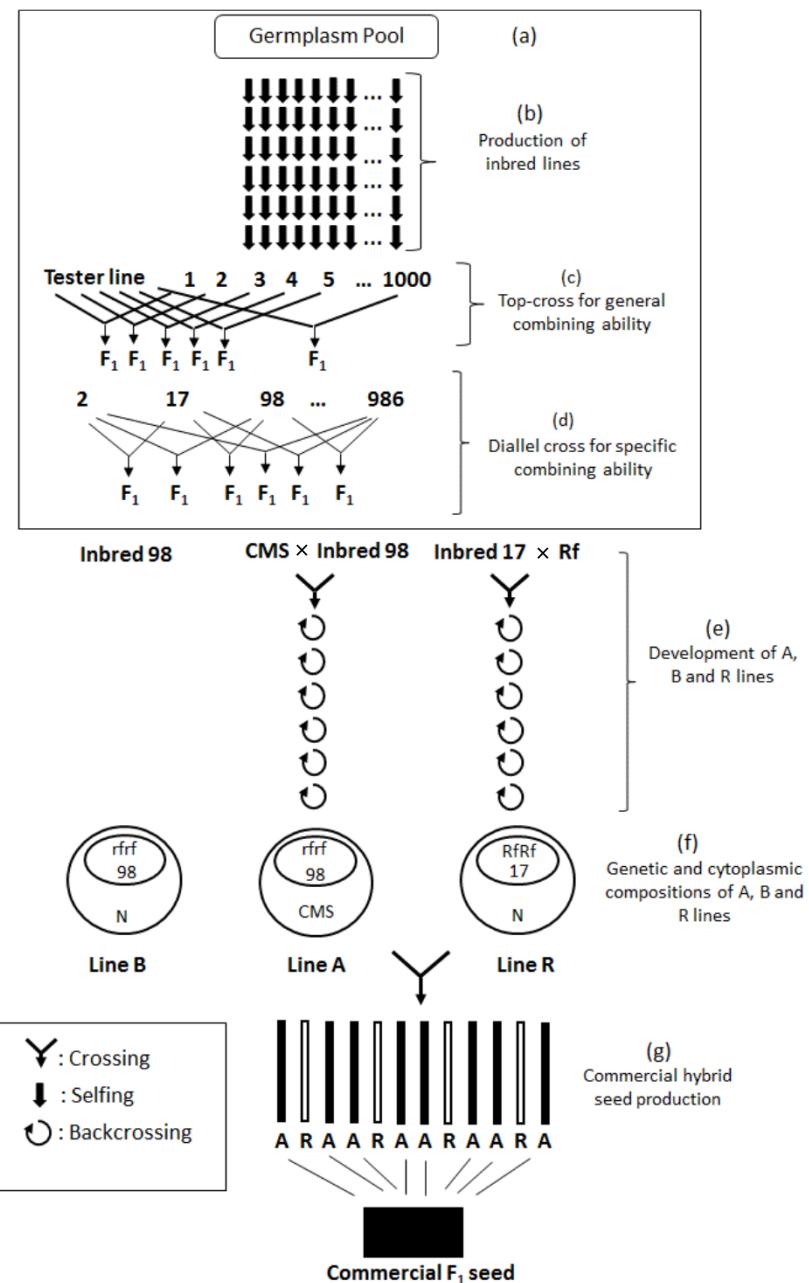


Figure 1. Scheme of classical hybrid variety development. **a)** A germplasm pool consisting of hybrids, open pollinating lines, or inbreds is established. **b)** Inbred lines are produced by 6–8 generations of selfing. **c)** Using a tester line, general combining ability (GCA) of the inbred lines is determined by growing hybrids in field conditions; about 50 inbred lines are selected based on GCA. **d)** Diallel crosses are made in all combinations among selected inbred lines and crosses are grown in field conditions to select the best one(s). Here, inbred lines 17 and 98 are selected. **e)** A, B, and R lines are produced by transfer of cytoplasmic male sterility (CMS) and fertility restorer genes (Rf) via six generations of backcrossing. **f)** R line is inbred line 17 with fertility restorer gene(s) and used as male in hybrid seed production; A line is inbred line 98 with CMS cytoplasm and used as female in hybrid seed production. B line is inbred line 98 and used for maintaining the A line, which is male sterile and cannot be multiplied. **g)** Commercial seed is produced in the field using A and R lines and only seeds from A lines are harvested as F_1 seed. Apomictic hybrid development, on the other hand, is completed at stage d within the upper frame and seed is multiplied as a seed clone.

sources and increasing their genetic vulnerability due to the cytoplasmic uniformity of all hybrids (Spillane et al., 2004). Modern agriculture needs fast adaptation to changing market and environmental conditions including changes in pest profiles. The shorter procedures and lower costs involved in apomictic hybrids will make it possible to adapt a “boutique breeding” approach to develop specific hybrids for microproduction areas (Jefferson, 1994).

Hybrids have higher yield performance in all crop species, whether self- or cross-pollinating, but due to the lack of appropriate male sterility systems and cost of hybrid production, hybrid varieties are feasible in only a few crops and account for less than 1% of all world acreage (Longin et al., 2012). Apomixis technology will eliminate the need for male sterility systems and falling seed prices will allow production of hybrid varieties in all crops. Yield increases of 20%–50% can be expected from hybrids in self-pollinating major crops such as rice and wheat (Tester and Langridge, 2010) as a result of apomixis technology.

Another advantage of apomixis in self-pollinating crops is that it will allow the development of new varieties

with one cross. There will be no need for six or seven selfing generations to make segregating loci homozygous (Figure 2). Since these crops are already pure lines, their F_1 will be similar to hybrid varieties developed using inbred lines. Thus, the whole variety development process, including seed multiplication and performance tests, will be completed within 3 or 4 years rather than 8 to 10 years.

Autonomous apomictic crops will not need pollination and hence do not require male system development. Therefore, elimination of male flowers or flower parts will save some precious assimilates in some plants (e.g., maize). Lack of need for pollination in autonomous apomictic crops will also prevent losses due to pollination failure, which has been gaining importance in recent years due to “pollinator decline” in cross-pollinating species (Kluser and Peduzzi, 2007) and threats to sexual reproduction caused by global warming (Hedhly et al., 2008). Pollination is a problem during hybrid seed production in some self-pollinating crops such as wheat and barley due to cleistogamy. Even when a cytoplasmic male sterility system is used, pollen from father plants cannot reach the stigma since the flower

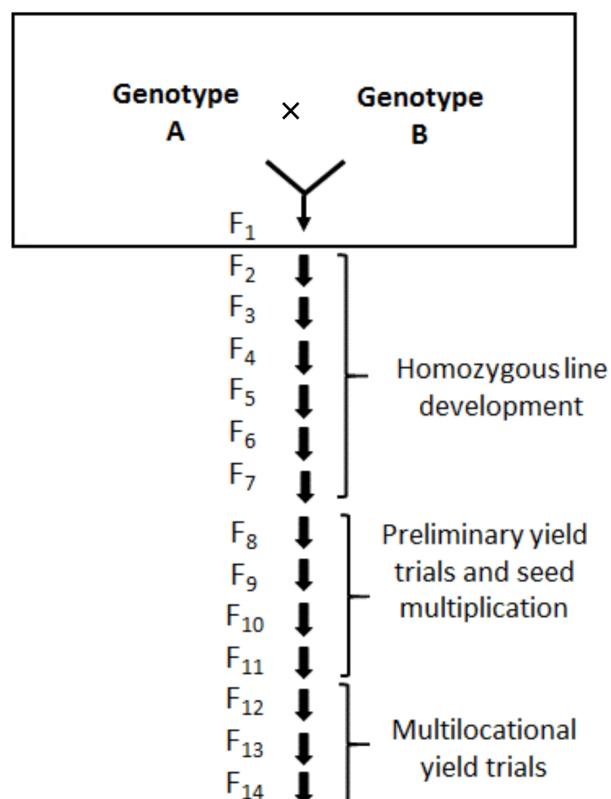


Figure 2. Scheme of a classical pure-line cultivar development in a self-pollinating crop. In F_2 – F_7 generations, all loci are made homozygous. Selections are made only for qualitative traits. Seeds of the homozygous lines are multiplied and evaluated in preliminary and then multilocational yield trials in F_8 – F_{14} generations. In apomictic self-pollinating hybrids, development of a new variety is completed in F_1 (upper frame), and seed of the produced line is multiplied and preliminary and multilocational yield trials are carried out.

is not opened. These plants need to be cross-pollinated for hybrid seed to be produced (Abdel-Ghani et al., 2004). Apomictic hybrids of self-pollinating crops do not have such a problem.

A major advantage of apomixis for plant breeding is that it will increase the survival of interspecific crosses since chromosomal irregularities in meiosis, and consequently hybrid sterility, is not a problem in apomictic plants. This could enormously increase the genetic diversity to be used in plant breeding. Apomixis is known to facilitate the survival of hybrids from wide species, at least under natural conditions (Bashaw and Hanna, 1990). Thus, apomixis might facilitate crosses with wild relatives in secondary and tertiary gene pools and offer new genes in those pools to the service of plant breeding (Spillane et al., 2004).

Apomixis will allow multiplication of clonal propagation material in the form of seeds in crops such as potato (Spillane et al., 2004) and cassava (Freitas and Nassar, 2013). Clonal propagation materials such as tubers, corms, or cuttings have serious disadvantages in terms of disease contamination, especially viral diseases. Making the propagation material free of diseases necessitates use of cumbersome tissue culture techniques. On the other hand, seeds carry much fewer plant viruses. For example, out of 16 disease-causing potato viruses, only two (potato spindle tuber viroid and potato virus T) have been proven to be carried by seeds (Salazar, 1996). Therefore, use of apomictic seeds as multiplication material instead of plant parts is expected to diminish the severity of viral disease and increase germplasm distribution throughout the world.

Clonal propagation is carried out using expensive and time-consuming tissue culture techniques. In some ornamental plants, efficient propagation is not possible due to the lack of suitable tissue culture protocols. In other plants such as potato, the propagation rate is not high enough to produce the planting material needed for large production areas. A typical multiplication rate for potato is about 10, which means that 8 or 9 cycles of potato tuber multiplication are needed to grow enough planting materials for a production area of 10,000 ha. Use of tissue culture methodologies for micropropagation and for minituber production can only slightly alleviate the problem. Potato is a plant that can produce plenty of seeds under suitable conditions, and when true potato seed is used as planting material, a few cycles of seed propagation will be enough to produce seed to plant large production areas.

The amount of clonally propagated crops that are used for planting constitutes great sums in some crops. For instance, about 10% of the potato crop is used for planting material. The use of true seeds in potato and cassava will

lead to the saving of 3.2 billion dollars' worth of tubers annually (Spillane et al., 2004). In addition, storage and transportation of live and bulky planting material adds to the planting costs in clonally propagated crops. Use of apomictic true seeds will lower seed cost and make the storage of planting material easier in these crops.

6. Concerns about the use of apomixis in crop species

As in the introduction of all advanced technological developments, there are some concerns about the use of apomixis in crops. The first is that some believe apomixis has not been able to meet the expectations so far. This is partly true, but until now apomixis has been transferred only using sexual hybridization, which has failed as mentioned earlier. Use of much more potent transgenic technology is more promising. Cloning of apomixis genes, a prerequisite for genetic transformation, is underway. In transgenic technology, a combination of different genes responsible for different stages of apomixis (i.e. apomeiosis, parthenogenesis, and autonomous endosperm development) could be used to get the best results for plant breeding purposes.

A second concern about apomixis is that this technology will cripple the seed industry by allowing farmers to produce their own seeds, jeopardizing future yield increases. This idea is completely baseless. Any kind of plant seed needs to be renewed at least every 5 years to protect the genetic and physical purity of the seed, to guarantee that seed is free from diseases and weed seeds, and to renew the variety used. The same will be valid for apomictic seeds. Presently, hybrid seeds are used in less than 1% of world production areas. When apomictic seeds have been introduced, all production areas of any crops will be accessible for hybrid seeds, since apomictic hybrids will be produced for all crops and their seeds will be cheaper than the present ones. Decreasing hybrid development costs will make development of hybrid cultivars for local areas feasible. All these factors will promote the seed industry rather than crippling it.

Another concern about apomictic crops is that apomixis genes could escape into wild relatives and cause genetic erosion. Various natural crossing barriers, especially ploidy differences, are thought to prevent such an escape between natural apomicts and sexual forms of the same species (van Dijk and van Damme, 2000). However, there is concern for such transfers from apomictic crops to sexual wild relatives, both of which might have the same ploidy level. Unlike the transgenes used in available transgenic crops, such as the Bt toxin and herbicide resistance gene, for which no selection advantage is present under wild conditions, the dominant transgenes of apomixis might have inherent reproductive advantages of apomixis (van Dijk and van Damme, 2000).

In addition, interspecific crosses formed as a result of gene escape from apomictic crops into wild relatives will not have meiotic irregularities leading to sterility and therefore will survive under natural conditions (van Dijk and van Damme, 2000). Although there are such theoretical risks, no report is available documenting genetic erosion in species that have apomictic and sexual forms of the same ploidy level. Furthermore, refining apomixis technology in the form of conditional apomixis, producing male sterile autonomous apomictic crops or maternal inheritance by placing the transgene in chloroplast, will easily obliterate these theoretical risks (Daniell, 2002).

7. Conclusions and future directions

Apomixis was a major obstacle that made life harder for plant geneticists. It even caused the termination of the career of Gregory Mendel, founder of modern genetics. The complex, multiple mechanisms of apomixis have been difficult to elucidate so far. However, due to its potential advantages in plant breeding, apomixis has been the focus of an enormous amount of investigation. Both natural

apomixis genes and mutations that break down the default sexual pathway and divert it to apomixis have been investigated. As a result, it has been possible to achieve at least some components of apomixis artificially. Research has shown that apomixis can be realized by different approaches in each of its three major stages: apomeiosis, parthenogenesis, and seed formation. A combined approach that makes use of different mechanisms might be necessary to take full advantage of apomixis while eliminating various concerns about its use in plant breeding.

Use of apomixis could change the face of plant breeding in the near future. First of all, use of hybrid seeds in almost all crops, including self-pollinating and clonally propagating ones, could be feasible. In addition, decreasing seed costs will make hybrid seeds accessible for poor farmers in low yielding areas. Moreover, most plant breeding programs could be completed with one cross, which is a big advantage for plant breeding in an era that necessitates quick responses to changes in environmental and marketing conditions.

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