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Apomixis: The Molecular Perspectives and its Utilization in Crop Breeding

YASIN J KHAN* RAVISH CHOUDHARY[1] HUSNARA TYAGI[2] AND ANIL K SINGH[3]

Divison of Genomic Resources, NBPGR, PUSA campus, New Delhi, India

ABSTRACT

Apomixis gives rise to a novel prospect in agricultural production globally by establishing genetically stable, seed-propagating clones of crops, which can produce numerous sporophytic true-to-type generations. This asexual mode of reproduction may prove to be an unrivalled crop improvement tool to achieve higher yields and along with highly desirable traits in modern agriculture. In fact, apomixis results in offspring that are exact genetic replicas of its single parent because embryos are derived from the parthenogenic development of apomeiotic egg cells. The development of crops with apomictic ability to produce true to type progenies in agriculture requires a deeper knowledge of the mechanisms regulating reproductive development in plants. Molecular understanding of apomixis would be greatly increased if genes that are specifically or differentially expressed during the formation of the embryo and embryo sac could be identified and transgressed in cultivated crops

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INTRODUCTION

Apomixis, refers to the occurrence of an asexual reproductive process in the place of normal sexual processes involving reduction division and fertilization; first identified by Leuwenhock in citrus In other words apomixis is a type of reproduction in which reproductive structures take part but seeds are formed without union of gametes. Seeds formed in this way are of vegetative origin. In obligate apomictic crops, apomixis is the only method of reproduction whereas facultative apomicts found to have coexistence of gametic and apomictic seed production. Apomixe is the substitution for sexual reproduction or another asexual reproductive process that does not involve nuclear or cellular fusion (*i.e.* fertilization). More than 300 species belonging to 35 families are apomictic (Bashaw and Hanna, 1990). It is most common in *Gramineae*, *Compositae*, *Rosaceae* and *Rutaceae*. Among the major cereals, maize, wheat and pearl millet have apomictic relatives. Angiosperms generates genetically diverse progeny because of the recombination and independent assortment of genes derived from both parents and entails a series of developmental steps which leads to the formation of the seed (Yang and Sundaresan, 2000). The process is highly regulated and most aberrations result in abortion (Yang and Sundaresan, 2000). Nevertheless, plants have retained considerable plasticity in the possible outcomes of reproduction. Apomixis therefore allows perpetuation of a fixed genotype through generations. The ability to fix indefinitely even highly complex genotypes, including high yielding hybrids, through apomixis would have tremendous advantages in plant breeding and seed production. Apomixis usually occurs in the wild species, hence poses the scope for various crop improvement programs. The genetics of apomixis various species has been under scrutiny for several decades, with the aim of transferring apomixes to their crop relatives. These studies so far have revealed that the developmental mechanisms underlying apomixes are complex; most genetic analyses suggest that apomixis is inherited as a simple Mendelian trait, and results from one or a few mutations that affect the normal

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[1] IARI Regional station, PUSA, Bihar, India
[2] IARI Regional station, Kalimpong, West Bengal, India
[3] Division of Land and Water Management, ICAR Research Complex for Eastern Region, Patna, Bihar, India

* Corresponding author email: yasinlab.icar@gmail.com

course of sexual reproduction (Savidan, 2000). The growing popularity of apomixis research has attracted developmental biologists into the field. The common perception is that the apomixis is not a true novelty in plant development, but rather has evolved through the rearrangement of the sub-programs that constitute a normal sexual pathway (Grossniklaus, 2001). In contrast to sexual reproduction, seedlings arising from apomixis retain the genotype of the maternal parent. The transfer of apomixis and its effective utilization in crop plants has major advantages in agriculture. However in the era of genomics, achieving an understanding of the genetic control and molecular regulation of apomixis appears complicated. Large amounts of cytological and ecological information along with genetic and molecular data, have been collected mainly from model species (viz., *Boechera holboellii, Hieracium* spp., *Hypericum perforatum*, *Paspalum* spp., *Poa pratensis, Ranunculus* spp., and *Taraxacum officinale*) and have often been tested in *Arabidopsis thaliana* (*Arabidopsis*) to elucidate the mechanisms of apomeiosis, parthenogenesis and apomixis) (Barcaccia and Albertini, 2013). The apomixis includes apomeiosis, parthenogenesis, and developmental adaptations to ensure functional endosperm formation. Apomixis and sexuality are closely interrelated developmental pathways where apomixis can be viewed as a deregulation of the sexual process in both time and space (Koltunow and Grossniklaus, 2003).

Types of apomixis

Mainly four types of apomixis.1. Recurrent Apomixis, 2. Non -recurrent Apomixis 3. Adventive or/ Poly embryony 4. Vegetative apomixes. According to Chaudhury *et al.* (2001), three types of apomixis are generally recognized *viz.*, diplospory, apospory and adventitious embryony. In sexual reproduction the megaspore mother cell (MMC) undergoes meiosis, spore selection (SS), and mitosis to produce the common eight-nucleate polygonum-type embryo sac (PESc). The embryo and endosperm compartments of the seed initiate development after double fertilization (DF) and give rise to mature seed (MS). In the panel showing diplospory, the Antennaria type is depicted where the MMC undergoes mitosis to form the embryo sac and endosperm and embryo production are autonomous. In other diplosporous plants fertilization of the polar nuclei (pseudogamy) is necessary for endosperm production. In apospory, the initials (AI) differentiate near the MMC, which may or may not have undergone meiosis. Apospory is the most common mechanism of apomixis in higher plants. Occasionally, it can be characterized by the presence of multiple embryo sacs. Sexual and aposporous embryo sacs may coexist or the sexual embryo sac may degenerate during formation. The aposporous embryo sac may degenerate during formation. The aposporous embryo sac can differ in structure to that in sexual species, as shown by the four-nucleate Panicum-type (P) as illustrated in fig. 1. Seed formation may be autonomous or pseudogamous. Polyembryony (PE) may be evident if multiple embryo sacs form. In adventitious embryony, numerous adventitious initial cells (AVI) differentiate next to an embryo sac derived by sexual processes. After double fertilization of the sexual embryo sac the numerous developing adventitious embryos gain access to the nutritive endosperm and seed contains multiple embryos (Fig. 1).

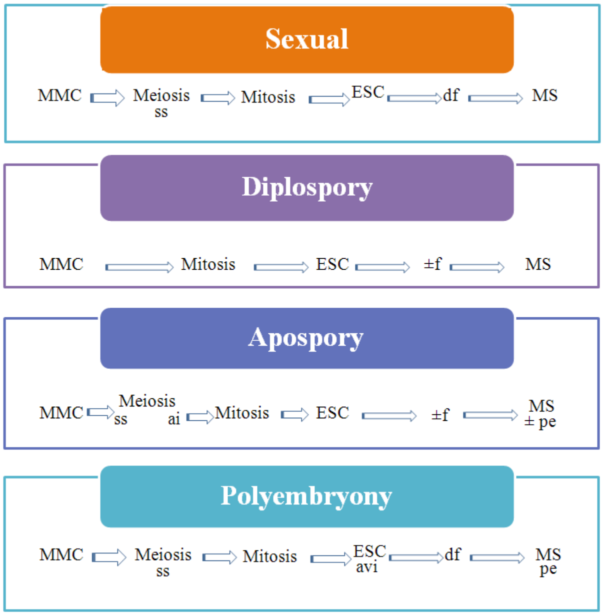

Fig. 1: Apomictic reproduction compared to sexual reproduction in flowering plants

On the other hand, gametophytic apomixis, the maternal embryo originates from a diploid egg cell differentiated in an unreduced embryo sac (Nogler, 1984). The unreduced embryo sac may arise from a somatic nucellar cell that acquires the developmental program of a functional megaspore and known as Apospory. Instead, in the diplospory pathway, the embryo sac originates from a megaspore mother cell with suppressed or modified meiosis. Apomictic plants may or may not change meiosis itself, but in any case they do activate the gametic cell fate either in a somatic cell (apospory) or in an unreduced megaspore (diplospory) as surrogate for meiotic products. Once apomeiosis (when 2n female gametophytes and gametes are formed) occurred, they subsequently undergo somatic parthenogenesis where embryogenesis autonomously without fertilization by a male gamete. Endosperm formation may be fertilization-independent (autonomous endosperm) or may require fertilization (pseudogamous endosperm). Among others, apospory has been reported in *Beta, Brachiaria, Cenchrus, Chloris, Compositae, Eriochloa, Heteropogon, Hieracium, Hyparrhenia, Hypericum, Panicum, Paspalum, Pennisetum, Poaceae, Ranunculus, Sorghum, Themeda*, and *Urochloa*, whereas diplospory has been noted in *Agropyrum, Allium, Antennaria, Boechera* (formerly Arabis), *Datura, Eragrostis, Erigeron, Eupatorium, Ixeris, Parthenium, Paspalum, Poa, Taraxacum*, and *Tripsacum* (Barcaccia and Albertini, 2013).

Apomixis in crop improvement

For commercial agriculture, plants have been selected from the excess of evolved flowering plant species, and wild species continue to be used as sources of genes in breeding and molecular crop improvement programs. Modern agriculture is dependent on uniformity of seed and fruit quality. This demand for product consistency in plantations of often non-genetically uniform populations is clearly at odds with the sexual reproductive mechanisms that have evolved to maximize genetic diversity. Sexual reproduction in agricultural systems also presents vulnerabilities associated with fertilization mechanisms. Fertilization is problematic if flowers are self-incompatible or if plants have unisexual flowers, since there is dependence on the simultaneous flowering of a compatible variety and on pollinator behavior mechanisms that are in turn influenced by environmental conditions and farm design. Thus, if crops could be produced independent of pollination and fertilization, these problems could be avoided, leading to more control in the consistency of crop yield. Clonal reproduction through seed, independent of fertilization, is not imaginary in plants. Some angiosperms commonly reproduce in this manner by a process called apomixis. Apomixis is an asexual method of reproduction through the seed, provides unique opportunities for developing superior cultivars in the future. It occurs at low levels in some cultivated species and can be found in closely related species of many cultivated crops. Transfer of apomixis to important crops would make possible development of true-breeding hybrids and commercial production of hybrids without a need for cytoplasmic male sterility and high cost, labor-intensive processes. Obligate apomictic hybrids would breed true regardless of heterozygosity. It could also provide an efficient method for incorporating genes into new genotypes and to determine genetic stability. The commercial hybrid production process would be simplified with apomictic hybrids (Hanna and Bashaw, 1987). The purpose of this review is to help plant breeders and geneticists to recognize apomixes, genetic basis, and use of apomixis in agricultural production, to discuss molecular approaches that may lead to the isolation of genes controlling apomixis to lock this process into elite production lines of agriculturally important crops, and to show how it can be used in plant improvement. Positive evidence for the presence or absence of apomixis can be obtained only from an intensive screening of a large number of plants in a variety/hybrid. The screening involves a careful and systematic tracing of steps for the development of embryo-sac and embryo, through microtomy of ovule, right from megaspores to embryonic developments. It should however be noted that it is only recurrent apomixis, namely diploid forms of apospory / parthenogenesis / apogamy / adventive embryony and vegetative propagation which are beneficial for plant breeding purposes. The simple reason being that, it produces viable diploid embryos without fertilization and thus, can continue to perpetuate over generations. Non-recurrent apomixis is of academic use only.

Advantages of apomixis in plant breeding

The two sexual processes, self-and cross-fertilization, followed by segregation, tend to alter the genetic composition of plants reproduced through amphimixis. Inbreeding and uncontrolled out breeding also tend to break heterozygote superiority in such plants. On the contrary, apomicts tend to conserve the genetic structure of their carriers. They are also capable of maintaining heterozygote advantages generation after generation. Therefore, such a mechanism might offer a great advantage in plant breeding where genetic uniformity maintained over generation for both homozygosity (in varieties of selfers), and heterozygosity (in hybrids of both selfers and outbreeders) is the choicest goal. Additionally, apomixis may also affect an efficient exploitation of maternal influence, if any, reflecting in the resultant progenies, early or delayed because it causes the perpetuation of only maternal individuals and maternal properties due to prohibition of fertilization. Maternal effects are most common in horticultural crops, particularly fruit trees and ornamental plants. Thus, in short the benefits of apomixis, insofar as their utility in plant breeding is concerned, are: (1) Rapid multiplication of genetically uniform individuals can be achieved without risk of segregation, (2) Heterosis or hybrid vigour can permanently be fixed in crop plants, thus no problem for recurring seed production of F_1

hybrids (3). Efficient exploitation of maternal effect, if present, is possible from generation to generation. 4. Homozygous inbred lines, as in corn, can be rapidly developed as they produce sectors of diploid tissues and occasional fertile gametes and seeds. Hanna and Bashaw (1987) also described how apomixes could be used in plant improvement. Apomixis would make it possible to fix the genotype of a superior plant variety bred for a particular environment or market niche so that clonal seeds faithfully representing that genotype could be continuously and cheaply produced independent of pollination. Additionally, the production of clonal seed is not only important for seed-propagated crops, but also for the propagation of heterozygous fruit tree crops and plantation timbers. Clonal seed would help avoid costly and time-consuming vegetative propagation methods that are currently used to ensure the large-scale production of these crops. Hybrid cultivars are widely used in agricultura1 production. They are the first-generation progeny (F1,) between two genetically different plants or inbred lines. F1, hybrid plants are heterozygous, normally uniform within the group, and may exhibit hybrid vigour, which is a sought after feature. Hybrid cultivars cannot be used as seed sources from next generation (F2,) because this generation would be extremely variable as a result of genetic segregation. Hybrid vigour is also reduced in the F2, generation. Therefore, parental stocks for hybrid seed production need to be maintained and the cross must be continuously repeated. Control of apomixis would enable the fixation of hybrid vigour and the development of true-breeding hybrids in a particular breeding program. Seed could be produced for many generations without loss of vigour or genotype alteration. Hybrid seed production would be simplified because line isolation would not be necessary to produce seed or to maintain parental lines, and the use of male-sterility lines could be avoided. Outcross contamination in hybrid seed programs lacking good male-sterility lines would also be eliminated. Overall, apomixes would enable a significant reduction in hybrid seed production costs. Apomixis could naively be viewed as a threat to the current viability of hybrid seed companies because farmers could process and plant their own seeds (Hanna and Bashaw, 1987). However, the current use of plant variety. The potential of genetically modified (GM) crops to transfer foreign genes through pollen to related plant species has been cited as an environmental concern. Until more is known concerning the environmental impact of novel genes on indigenous crops and weeds, practical and regulatory considerations will likely require the adoption of gene-containment approaches for future generations of GM crops. Most molecular approaches with potential for controlling gene flow among crops and weeds have thus far focused on maternal inheritance, male sterility, and seed sterility. Several other containment strategies may also prove useful in restricting gene flow, including apomixis, cleistogamy, genome incompatibility, chemical induction/deletion of transgenes, fruit-specific excision of transgenes, and transgenic mitigation (Daniell, 2002).

Apomixis: an alternative system of seed production

Apomixis occurs throughout the plant kingdom (Grossniklaus, 2001). Among angiosperms, more than 300 plant species from more than 35 families have been described as apomictic, with a distribution pattern that indicates a polyphyletic origin. The well-represented families are the *Gramineae, Compositae*, and *Rosaceae* (Richards, 1986), although this bias may be more reflective of research effort than natural abundance. Apomictic processes occur in the ovule, resulting in progeny that are genetically exact copies of the female plant because fertilization is redundant to produce an apomictic embryo. The apomictic embryo is formed in the ovule using two different pathways, sporophytic and gametophytic, which define the origin of the apomictic embryo (Gustafsson, 1946 and Koltunow, 1993). In sporophytic apomixis, the embryo arises directly from the nucellus or the integument of the ovule in a process generally called adventitious embryony (Naumova, 1993). In gametophytic apomixis, two mechanisms are usually documented which are diplospory and apospory. In both of these an embryo sac is formed and the two mechanisms are distinguished by the origin of the cells that give rise to the apomictic embryo sac. In diplospory, the embryo sac originates from megaspore mother cells either directly by mitosis and/ or after interrupted meiosis. In apospory, the embryo sac originates from nucellar cells (Gustafsson, 1946; Koltunow, 1993). Apomixis fixes a particular genotype because meiosis is not necessary to produce an embryo sac or an egg-like cell; therefore, there is no opportunity for recombination. Apomixis eliminates the need for events considered essential for the successful completion of reproduction by seed: meiosis is uncoupled from both female gametophyte development and egg-cell formation, and double fertilization is uncoupled from embryo and endosperm development (Koltunow, 1993).

Genetics of gametophytic apomixis

Consequently, cytological, molecular and ecological information have been collected mostly from *Boechera holboellii*, *Hieracium* spp., *Hypericum perforatum*, *Paspalum* spp., *Poa pratensis*, *Ranunculus* spp., and *Taraxacum officinale* model species and have frequently been tested in *Arabidopsis thaliana* to reveal the technology of apomeiosis, parthenogenesis, and apomixis (Barcaccia and Albertini, 2013). Several genes were responsible for the autonomous development of the embryo and endosperm along with unreduced embryo sacs and egg cells formation have been cloned and distinguished; nevertheless, none of these genes are capable of representing the apomictic pathway as a whole in crop plants. More recently, merging lines of evidence regarding the role of auxin in cell fate specification of the embryo sac and egg cell development have been reported in *Arabidopsis*. Apomictic plants produce progeny that are an exact genetic replica of the some higher mother plants. The replication is achieved through changes in the female reproductive pathway such that female gametes develop without meiosis and embryos develop without fertilization. During apomeiosis, when 2n female gametophytes and gametes are formed, they consequently go through somatic parthenogenesis by embryogenesis autonomously without fertilization through male gamete. Endosperm formation may be autonomous or pseudogamous endosperm (Barcaccia and Albertini, 2013). Apomixis might rely more on a global deregulation of sexual reproductive development than on truly new functions and molecular mechanisms. This new perspective has straight consequences for the apomixis engineering in sexual crop species, and could have an immense impact on agriculture (Grimanelli *et al*., 2001). Gametophytic apomixis is consideration to rely on three genetically independent Mendelian loci, each of them applying control over a key developmental component, including apomeiotic megaspores, parthenogenic egg cells, and modified endosperms (Grossniklaus *et al*., 2001; Koltunow and Grossniklaus, 2003; Bicknell and Koltunow, 2000). Initially, it was proposed a single regulatory gene for promoting apomixis. Although, molecular evidence suggested that a more complex inheritance system directs the entire process of apomixis. Linkage groups which contain large blocks of sequences typically transmitted with apomixis that lack genetic recombination between molecular markers in some species and leading to the expression of apomixis. The interesting opportunity is that DNA structure and/or RNA interference could regulate the expression of apomixis-related genes due to retrotransposons rich regions which were present in apomictic and heterochromatic region of genome has evaluated. Small regulatory RNAs regulate gamete function and fertilization in plants by changing gene expression through post-transcriptional gene silencing, translational inhibition, and heterochromatin modification in eukaryotes.

Polygenic inheritance or monogenic

Hereditary investigation in numerous species has consistently confirmed that a simple inheritance scheme involving a small amount of Mendelian genes which controls the apomixis mechanism and its expression of components. Apomixis is a heritable trait; however its genetic control is ambiguous (Nogler, 1984). It is found almost entirely in polyploid, highly heterozygous and genetically inadequated species, making its genetic itemization complicated (Koltunow, 1993; Richards, 1997; Savidan, 2000). Apomeiosis is the facet of apomixis that has attracted the most attention for the development of female gametes without meiosis The inheritance rudiments comprise a departure in the integer and role of genes, and relationships amid alleles, in addition to dominance of apomixis more than sexuality (Grimanelli *et al*., 2001; Koltunow and Grossniklaus, 2003). In contrast, molecular and cytogenetic analysis of chromosomal region(s) hauling the apomixis in several species that is likely based on a system of polygenes have exposed in addition to mechanisms, like lack of recombination, gamete elimination from trans-acting elements, supernumerary chromatin structures, and DNA rearrangements. From an evolutionary approaches, in several model species namely, *Poa Taraxacum* and *Hypericum*, *Erigeron Hieracium* and *Panicum maximum*, apomixis determined and included a narrow euchromatic region where genetic recombination between apomeiosis and parthenogenesis loci, and their linked genes was possible. Whereas, apomixis determination with large non-recombining regions surrounding the apomixis locus due to a degenerate heterochromatic block which carrying apomixis factors in *Pennisetum/Cenchrus*, *Brachiaria Paspalum* and *Tripsacum* (Grimanelli *et al*., 1998) model species. Recently, recombination in between apospory and parthenogenesis loci was found in *C. ciliaris*. They revealed that the number of genes involved in the expression of genes which controls apomeiosis, parthenogenesis and eventually autonomous endosperm development. In contrast, transposable, repetitive and pseudogenes elements within the chromosome block carrying apomixis genes create a complex genetic system, with loci that show a discrepancy from elementary and primitive to evolutionarily advanced.

Table1: Basic information on molecular basis of apospory, diplospory and polyembryony in apomictic plant species

Plant Species	**Types of apomixis**	**References**
Beta	Apospory	Cleij *et al.* (1976)
Chloris	Apospory	Barcaccia and Albertini (2013)
Compositae	Apospory	Barcaccia and Albertini (2013)
Eriochloa	Apospory	Barcaccia and Albertini (2013)
Heteropogon	Apospory	Barcaccia and Albertini (2013)
Hieracium	Apospory	Koltunow *et al.* (2011)
Hyparrhenia	Apospory	Barcaccia and Albertini (2013)
Ranunculus	Apospory	Nogler (1984)
Sorghum	Apospory	Barcaccia and Albertini (2013)
Themeda	Apospory	Barcaccia and Albertini (2013)
Urochloa	Apospory	Barcaccia and Albertini (2013)
Agropyrum	Diplospory	Barcaccia and Albertini (2013)
Allium	Diplospory	Barcaccia and Albertini (2013)
Antennaria	Diplospory	Barcaccia and Albertini (2013)
Boechera (formerly Arabis),	Diplospory	Barcaccia and Albertini (2013)
Datura	Diplospory	Barcaccia and Albertini (2013)
Eupatorium	Diplospory	Barcaccia and Albertini (2013)
Ixeris	Diplospory	Barcaccia and Albertini (2013)
Paspalum	Diplospory	Barcaccia and Albertini (2013)
Citrus spp	Polyembryony	Barcaccia and Albertini (2013)

Polyploidy

Apomeiosis is inherited as a single dominant Mendelian trait in a forage grass (*Panicum maximum*) and a dicotyledonous plant (*Ranunculus auricomus*), which are tetraploid. This result as evidence for monogenic inheritance and recommends a functional relationship between the traits also suggested that a Mendelian trait can cover anything from a single gene to an entire chromosome (Bicknell *et al.,* 2000). This complexity of the regulatory pathways needs to be altered for the development of apomictic seeds. Overall, apomixis might require the coordinated deregulation of several genes for reproduction. Polyploidy is a probable path for such type of deregulation. It seems that complete or segmental polyploidy is ever-present in gametophytic apomicts. Previously, *Arabis holboellii* are diploid apomicts, but after the *Arabidopsis* genome sequenced those might well be paleopolyploids. Apomixis might eventually be expressed in diploids, but that alleles are not transmitted by haploid gametes, or may be fatal in diploid progenies (Grimanelli *et al.,* 1998; Tas and van Dijk, 1999). These researchers proposed the relationship between apomixis and polyploidy and revealed that might be structural more willingly than functional.

Apomixis and genomic imprinting

Genomic imprinting refers to parent-of-origin specific gene expression, and it provides maternal and paternal genomes differ to each other functionally and may affect entire genomes, chromosomes or individual loci (Tilghman, 1999). Mammals do not have apomixis-related trend due to genomic imprinting renders the maternal and paternal genomes complementary for genes that are essential to embryo development (McGrath and Solter, 1984), and thus it ensures that both genomes are present in the zygote. Apomixis is relatively recurrent in flowering plants (Savidan, 2000). The relatively frequent occurrence of apomixis indicates that embryo development in plants must be managed by radically different rules. Vielle-Calzada *et al*. (2000) gave a promising justification after survey of 20 genes in *Arabidopsis thaliana* and revealed that none of the paternally derived transcripts were detectable in the embryo or endosperm for the first few days after fertilization. This early phase of development under maternal control could be largely, before fertilization (combination of maternal products stored in the gametes) and after fertilization (uniparental expression of some genes) that is, genomic imprinting. Hence, the differentiation between apomictic and sexual seed development should lie in the mechanisms that regulate the activation of the correspondence. On the other hand, several regulatory factors have been identified in plants that induce partial embryo development or embryo-specific gene expression (Ogas *et al*., 1999).

Endosperm development in apomictic plants

The endosperm is similarly important to apomictic and sexual seed development, but its development significantly vary between the two pathways such as autonomous in *Erigeron* or *Taraxacum* (apomicts) in which endosperm development occurred by the partheno-genetically without fertilization, while in *Panicum*, *Pennisetum* or *Tripsacum*, depends on fertilization of the central cell by a male sperm known as Pseudogamous. Both these types of apomict fluctuate from their sexual counterparts in the maternal and paternal genomes. Endosperm development has hardly ever been considered by the inheritance of apomixis

(Nogler, 1984; Koltunow, 1993; Richards, 1997; Savidan, 2000). Genome dosage is critical to seed development in several plants species. Most other cereals especially in maize, normal endosperm development necessitates a maternal to paternal genome ratio of 2:1 which cause seed abortion (Birchler, 1993). This phenomenon is a strong obstruction to the emergence of apomixis and to introgression of the trait in crop species during evolution (Nogler, 1984; Savidan, 2000). In apomicts, the requirement is undisturbed (Quarin, 1999) or assembled by gametogenesis or fertilization (Nogler, 1984; Savidan, 2000; Grossniklaus *et al*., 2001). In many aposporous plants especially *Panicum*, have a modified embryo sac, in which the central cell contains only a single unreduced polar nucleus followed by fertilization to produce an endosperm with a 2:1 ratio. It seems that apomictic *Tripsacum* and *Paspalum* are moderately resistant to dosage variation (Grimanelli *et al*., 1997; Quarin, 1999) and suggested about the strict requirement for a genome ratio (2:1) in maize, not in all sexual plants. However in *Arabidopsis* seed viability is less susceptible to genome dosage ratio (Scott *et al.*, 1999) but in maize susceptibility to dosage effects in the endosperm with noticeable suggested that probably apomixis would create problems with seed viability. Introgression of apomixis between sexual crops from wild relatives has been typically attempted and as a result found high level of seed abortion due to the dosage constraints which were not transmitted with apomeiosis and parthenogenesis (Morgan *et al*., 1998). It is clear that genome dosage is an essential aspect of the genetics of apomixis while genetic bases of dosage response in the endosperm still unknown.

Molecular basis of apomixes

Genetic analyses suggested that the isolation of the gene(s) controlling apomixis might be feasible; however, although molecular strategies towards gene expression during apomictic reproduction remains inadequately implicit (Chaudhury and Peacock, 1994; Koltunow *et al*., 1995). Meiosis and egg cell fertilization are misplaced making offspring exact genetic replicas of the mother plant during apomictic processes. In an apomictic tropical forage grass (*Brachiaria*) gene expression in mature ovaries containing reduced and unreduced embryo sacs was compared to gain an imminent by the molecular basis of apomictic reproduction. Some limitations mainly from several differences in the genetic make-up of the plant material occurred due to experimental systems for the molecular study of apomixis and sexuality. Transcriptome data of various apomictic and sexual plants can overcome these limitations using the polymerase chain reaction (PCR) differential display and non radioactive cDNA detection procedures. Gene expression in pooled mRNA analysis is a potential tool to elucidate the molecular basis of apomixes (Leblanc *et al*., 1997). In contrast, apomixis research has focused on cyto-embryological mechanisms, population genetics (Marshall and Brown, 1981), inheritance of apomixis in various species and co-segregating markers (Leblanc *et al*., 1995).

Perspectives

The main approaches that have been followed to study the molecular basis of apomixis address the isolation of genes that show the expression of apomixis in natural apomicts and/or the identification of genes that express the features of apomictic pathways when they are deregulated in model sexual systems. Once an apomictic plant is detected its inheritance should promptly be studied by crossing a half or few flowers with the pollen obtained from normal plants and going through the segregation pattern in F2 and onward generations. The remaining flowers may thoroughly be checked and seeds collected on maturity. The true nature of such plants would become distinct only after progeny tests. A true apomictic plant will automatically produce mother apomictic progenies which can be maintained without difficulty. The introgression of apomixis into crop species from wild relatives failed mainly because natural apomicts are characterized by hybridity and polyploidy, and the loci controlling apomixis usually have a simple inheritance but a complex structure. In fact, these loci are apparently located in very large chromosomal regions, which make them recalcitrant to recombination-based genetic mapping strategies in addition to complicating their physical cloning. The greatest progress has been made with the introgression of apomixis into pearl millet from *Pennisetum*, where a single alien chromosome persists in the most advanced apomictic lines. Most importantly, the three main components of apomixis could not be successfully combined into a single model plant to date. Consequently, although findings in *Arabidopsis* represent the first steps toward the synthesis of an artificial apomixis system, the engineering of asexual reproduction in *Arabidopsis* has yet to be accomplished. Because there are a large number of candidates, it is likely that a cross-check between apomictic species should be carried out to assess both analogous and unique genes. In fact, if some of these genes are truly involved in apomixis, their functions should be conserved in other species. The link between apomixis and gene-specific silencing mechanisms, including chromatin remodeling factors or trans-acting and heterochromatic small interfering RNAs involved in both transcriptional and post-transcriptional gene regulation, is beginning to become clear. In fact, merging lines of evidence regarding the role of micro RNAs in the control of transcription factors, which act on genes directly involved in the development of embryo sacs, embryos, and seeds, have been reported in *Arabidopsis*.

CONCLUSION

Recent molecular studies aimed at understanding the basis of apomixis and so far it could not express the core idea of apomicts. Another shortcoming is that apomicts are not reported in agriculturally important crops and do not have agriculturally important relatives. Additionally, apomicts have not been sequenced so far, therefore genome annotation information is not available to support the research further. If zygotic embryogenesis (sexuality) and apomeiotic parthenogenesis (apomixis) follow similar pathways during embryo and seed development, it has to be considerable that specific genes have to be activated, modulated, or silenced in the primary steps of plant reproduction to ensure that functioning embryo sacs develop from apomeiotic rather than meiotic cells. Other genes could be specifically or differentially expressed in sexual and apomictic plants during embryo and endosperm development.

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