

Biology of Apomixes: Molecular and Genetic Approaches

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Introduction

Molecular and genetic aspects that lead to apomixis are discussed and reviewed. Key events result to altered regulation of female gametophyte development and subsequent embryo and endosperm development. In this paper, developmental aspects of sexual and apomictic reproduction are presented, including among other topics sporophytic and gametophytic relationships, embryo and endosperm development, interrelationships among sexual and apomictic mechanisms of reproduction, and models that explain apomixes are discussed.

Genetic control of apomictic reproduction is discussed basis on four related topics: megasporogenesis and nonreduction process, megagametogenesis, egg activation and parthenogenesis, and the endosperm development and genomic imprinting. Screening for mutants that display apomictic traits in a sexual species and could directly lead to the identification of key apomictic regulatory components, and the novel techniques of enhancer detection are reviewed, focusing on the generation of transposants and the identification of developmentally regulated genes and their promoters. Also, the introduction of apomixis into sexual species by means of the classical and *de novo* engineering of apomixes through biotechnology are retaken.

Finally, the conclusion includes topics such as the advances in relation to the engineering in apomixes, and its application in field conditions. One more issue to concern about apomixes is about the ethics in the use of its knowledge and technology, in relation to intellectual property rights, patenting, licensing, and availability to the public and private sectors worldwide.

Developmental Aspects of Sexual and Apomictic Reproduction

Apomixis is reported in more than 400 species belonging to 40 families. It occurs more frequently within the Asteraceae, Poaceae, and Rosaceae families. According to John Carman, only 127 of the more than 14,000 genera of flowering plants contain apomictic species (Spillane et al. 2001a). Altered regulation of female gametophyte development and subsequent embryo and endosperm development are likely to be responsible for apomixes (Chaudhury et al. 2001).

Types of apomixes

There are two types of apomixes:

1. Gametophytic apomixes, if the gametophyte originates from unreduced cells.

2. Sporophytic apomixes or adventitious embryony, if the unreduced cells form an embryo directly.

Gametophytic apomixes is subdivided depending on whether the megagametophyte is developed from an unreduced megaspore (diplospory) or from a sporophytic cell in the nucleolus (apospory). Different kinds of apomixes can co-exist within an individual plant (Spillane et al. 2001b).

Double fertilization

An important issue in sexual reproduction of an angiosperm's life cycle is seed development, which starts with the double fertilization phenomenon. In diploids, the female gametophyte is contained by the ovule. Contained within the ovule are a haploid egg cell in the micropylar end, a diploid central cell, two synergids adjacent to the egg cell, and three antipodal cells in the chalazal end (Chaudhury et al. 2001). When double fertilization occurs, both the embryo and the endosperm are obtained. The embryo is formed when the haploid egg is fertilized by one of the sperm cells and the triploid endosperm forms when the maternal homodiploid central cell is fertilized by a second sperm cell (Chaudhury et al. 2001). This process occurs inside the maternal ovule tissues, which are surrounded by the diploid sporophytic layers of inner and outer integuments (Chaudhury et al. 2001).

In contrast, apomictic plants by-pass or deregulate the sexual developmental program described above at various steps (Koltunow, 1993). Meiosis may be interrupted or absent resulting in an unreduced female gametophyte with the full complement of maternal chromosomes (apomeiosis). Fertilization may not occur and an autonomous embryo is produced (parthenogenesis). Endosperm development may be initiated autonomously or sexually. In the later case, embryo sac development or fertilization is often modified to adjust to a different genomic context (Grossniklaus et al. 2001; Naumova et al. 2001). With apomixis, only the female reproductive program is altered, pollen formation is usually unaffected in apomicts (Spillane et al. 2001b).

Sporophytic and gametophyte relationship

Sporophytic tissues of the ovule have maternal functions for the development of the seed, and contribute to the seed coat. During seed development, the seed depends and is related to the ovule and female gametophyte development before the fertilization occurs. Within the ovule primordium, the megasporangium produces the meiotic cells from which the female gametophyte is generated. The sporophytic cells that surround the female gametophyte express gene products required for female gametophyte development and function (Chaudhury et al. 2001). Apparently meiosis and female gametophyte development are controlled by regulatory genes that may be deregulated in space and time in apomicts (Koltunow, 1993; Grossniklaus et al. 2001b). Many events relevant to apomixes, such as the initiation of the egg cell and modified fertilization mechanisms are functions of the developing female gametophyte (Spillane et al. 2001a). Grossniklaus et al. (2001b) have identified mutants that were affected in female gametophyte development and double fertilization (Chaudhury et al. 2001).

Apomeiosis: Embryo development

Embryogenesis is the process in which the unicellular fertilized zygote changes in progressive way to the embryo. The main body parts of the embryo include the apical

meristem, hypocotyls, cotyledons, root, and root meristem. Around the apical basal axis, a radial pattern is established and it contains the epidermis in the periphery and layers of vasculature and conductive tissues within (Chaudhury et al. 2001). Embryogenesis is this same process, but occurs in apomictics completely without the contribution of the paternal genome (Spillane et al. 2001) and an unreduced diploid egg develops into an embryo without fertilization (Chaudhury et al. 2001).

Parthenogenetic embryogenesis

Pseudogamy is the pollination and fertilization of a portion of the ovule. There are two types of pseudogamy, in the first; pollination and fertilization of the central cell are needed for parthenogenetic egg development. In the second type, initiation of embryo formation is precocious and independent of pollination and central cell fertilization, but fertilization-induced endosperm development is required for seed viability. Precocious embryo development also provides a means to avoid fertilization of the unreduced egg cell.

Apomictic citrus species commonly produce many embryos that grow from the nuclear tissue of the ovule surrounding a fertilized female gametophyte. Digestion of the nucleus occurs as the sexually derived embryo and endosperm grow and expand within the nucleus, but the nuclear embryo initial cells are not digested, by this reason seed of the apomictic citrus are often highly polyembryonic (García et al. 2001). Successful fertilization of the sexually derived embryo sac is not required for the initiation and development of nuclear embryos in apomictic citrus. In seedless and also low-seeded citrus varieties that exhibit high rates of female gametophyte abortion, the nuclear embryos initiate near a region of nuclear cell degradation in the chalazal region of the unfertilized ovule, quite distinct from the aborted female gametophyte (Chaudhury et al. 2001).

Endosperm development

There are two hypotheses over the origin of endosperm:

1. One postulates that the endosperm forms from a twin embryo that assumed a growth support role.
2. The other hypothesis is that the endosperm seems to be the result from the extended development of the megagametophyte, which may be promoted when the central cell is fertilized by the second male gamete (Chaudhury et al. 2001).

Basic steps of endosperm development are conserved between monocots and dicots. Endosperm development in *Arabidopsis* and barley consists of four stages: syncytial, cellularization, differentiation, and death. Endosperm has as a main function to nourish the embryo; in cereals, the endosperm is a site of storage reserves, but in species such as carrot, endosperm is thought as a source of signals involved in embryogenesis (van Hengel et al. 1998).

Endosperm development in apomicts can be autonomous or it can require pollination and fertilization (pseudogamy). Pseudogamy is typical for Rosaceae and Gramineae families, and it is the most common mode of the endosperm development in the apomictic plants (Chaudhury et al. 2001). Some mutants affect endosperm development. For example in the *fis* class of mutants (*mea*, *fis2*, and *fie*) diploid endosperm is formed autonomously without fertilization, thus endosperm development is

suppressed by the wild-type function of these genes. Crosses between plants with different ploidy levels cause abnormal endosperm development, affecting successful hybridization (Chaudhury et al. 2001).

The maternal genome content of pseudogamous endosperm depends on the number of unreduced central cell nuclei fusing with the unreduced sperm cell nucleus. Autonomous endosperm production is rare in apomicts and is most common in the Compositae (Chaudhury et al. 2001).

Sexual-Apomictic Interrelationships

Most apomicts are facultative, that is both apomictic and sexual reproduction occurs at different level and even within the same plant. Obligate apomicts are those plants in which a sexual reproduction pathway is not present (Spillane et al. 2001b). There are species, such as *Poa pratensis*, in which its reproductive behavior ranges from nearly obligate apomixis to complete sexuality (Barcaccia et al. 1999).

Models of Apomixis

Apomixis can occur due to heterochronicity or heterotocity of the sexual developmental process of reproduction. Heterochronicity refers to mistakes in the timing of developmental processes, for example; megagametogenesis occurs before megasporogenesis is completed or embryogenesis starts prior to fertilization (Spillane et al 2001). Causes of the heterochronic development can be perturbation of developmental checkpoints or changes in gene expression due to polyploidization (Grossniklaus, 2001). Heterotocity is related to mistakes in the location where developmental events occur. It may be due to deregulation of cell fate specification (Grossniklaus, 2001). The formation of the embryo initials from integumentary or nucellar cells (adventitious embryony) and the formation of a gametophyte from nucellar cells (apospory) are examples of heterotocity effects in apomicts (Spillane et al. 2001).

Apomixis-polyploids relationships

Naumova et al. (2001) enumerates two hypotheses to explain the prevalence of apomixis among polyploids. According to the hybridization-derived floral asynchrony theory, apomixis arose from asynchronous expression of duplicate sexual reproductive gene sets in hybrid or polyploid genomes. Nogler (1984), cited by Naumova et al. (2001), postulates that the alleles responsible for apomixis may act as, or are linked to, recessive lethal factors, thus they can only be transferred by a diploid or polyploid gamete.

Polyploid crops must pair and segregate closely related chromosomes correctly during meiosis in order to maintain genetic stability and fertility. Homologous chromosomes in polyploids, must accurately distinguish each other (Spillane et al. 2001). Additionally, facultative apomixis is related with a high and irregular ploidy level and a high and variable level of heterozygosity (Barcaccia et al. 1999). Thus, polyploid apomixes is a difficult and precise process.

Genetic Control of Reproduction and Candidate Genes for the engineering of Apomixis

There have been no fully apomictic mutants recovered from sexual species to date. Only individual components of apomixis in several mutants and spontaneously occurring variations of sexual reproduction have been observed. These components consist of:

1. Production of unreduced spores.
2. Formation of parthenogenic haploids.
3. Autonomous activation of endosperm development.

It is believed that sexual and apomictic modes of reproduction share many genetic regulatory components. The engineering of apomixis will require a better understanding of these regulatory mechanisms, especially the female reproductive development in sexual plants at the molecular and genetic level. Until recently, ovule development has been the focus of genetic analysis of female reproduction. Thus, the genetic and molecular mechanisms controlling megasporogenesis, megagametogenesis, and fertilization is in need of more attention.

Megasporogenesis and Nonreduction

Megasporogenesis involves the determination of a megaspore mother cell, meiosis, and the selection and differentiation of the functional megaspore. Gametophytic apomixis involves production of an unreduced gamete and its parthenogenetic development either with or without the endosperm development. The important point to ponder here is what are the developmental checkpoints during megasporogenesis that can be simulated or overcome to induce an unreduced cell to initiate megagametogenesis.

Analysis of mutants affecting megaspore mother cell differentiation and meiosis may lead to an understanding of how to initiate apomixes during megasporogenesis. In some species multiple megaspore mother cells are found, suggesting that several nucellar can enter the meiotic pathway. Once a cell is committed, it inhibits neighboring cells from undergoing meiosis. This view is supported by analysis of maize mutant *multiple archegonial cell1 (mac1)*. Plants homozygous for *mac1* contain approximately three to 21 megasporophytes in a single ovule. Indicating that *mac1* is involved in megaspore mother cell determination (Sheridan et al. 1996).

The genetic regulation of meiosis has been extensively studied in maize and baker's yeast. Many genes playing an active role at different stages of meiosis have been identified. Genes acting early in the pathway and deciding between mitosis and meiosis are of particular importance. One important early regulator gene identified is *IME1*. *IME1* is required for the expression of the genes acting in the early phase of meiosis and is activated upon phosphorylation by *RIM11* (Kawaguchi et al. 1992; Bowditch et al. 1994). Upon activation by phosphorylation, the early genes are activated and diploid cell undergoes meiosis to produce four haploid spores. Thus, regulation involving phosphorylation and dephosphorylation events is required for meiosis to take place.

Meiosis is a universal phenomenon in eukaryotes. Therefore, the regulatory events are likely to be conserved at the molecular level. Identification of the homologs of the meiotic genes in yeast could be useful in the engineering of apomixis. In maize, mutants that influence the decision between mitosis and meiosis have been identified, but their molecular nature is not known. In plants homozygous for the *ameiotic1 (am1)* gene,

fail to undergo meiosis and show mitotic division (Palmer 1071). Thus this gene is likely to control the transition from mitosis to meiosis and is important for initiation of meiosis prophase1 (Golubovskaya et al.). Thus, the study of the molecular level of meiosis is an important phase of our understanding of apomixes.

Megagametogenesis

Megagametogenesis involves the formation of an embryo sac from the functional megaspore. Transmission studies of chromosomal deletions and deficiency analysis in maize have shown that a large number of loci essential for embryo-sac development are dispersed throughout the genome. Mutants have been produced by insertional mutagenesis and transposon based mutagenesis. The mutants *sre* are semi sterile and show non-Mendelian segregation.

The megagametophyte carrying *indeterminate gametophyte (ig)* mutant gene in maize, undergoes abnormal mitotic divisions and are transmitted through the female gametophyte at a reduced frequency (Huang and Sheridan 1996). Embryo sacs mutant for *lethal ovule (lo1 and lo2)* does not produce any viable seeds (Nelson and Clary 1952). The *lo2* embryo sacs have defective nuclear divisions and migrations and arrest predominantly at 1 and 2 nucleate stages. It has been observed that spatial and temporal coordination is required for proper embryo sac development. In *hdd* mutant gene embryo sacs, nuclear divisions at the micropylar and chalazal end are asynchronous and may show premature cellularization (Vollbrecht and Hake 1995). Thus *hdd* uncouples the tightly coordinated spatial and temporal cellularization.

Egg Activation and Parthenogenesis

The understanding of the events after fertilization has been limited by the inaccessibility of the gametes and the megagametophyte where double fertilization occurs. Recent developments have enabled us to study specific fusion of isolated maize gametes. One of the first visible changes that occur following fertilization is the formation of the cell wall. Cell wall formation is effected by a transient rise in the cytosolic calcium levels. This observation raises a question can increase in cytosolic calcium levels cause development of parthenogenetic egg?

The frequency of maternal haploids is increased fourfold in plants homozygous for *ig*, as compared to isogenic lines homozygous for the wild type *Ig* allele. The *haploid initiator (hap)* mutation causes extremely high frequencies of haploid production in barley plants. This mutation prevents the sperm from fertilizing the egg by an unknown mechanism whereas the central cell gets fertilized normally to produce the nutritive endosperm (Morgensen 1988).

Endosperm Development and Genomic Imprinting

The interactions between embryo, endosperm, and maternal tissue are a complex and poorly understood aspect of seed development. There is growing evidence of effects of endosperm development on apomixis may be linked to some of the following components:

1. Interrelationship of embryo and endosperm development.
2. Genomic imprinting.
3. Imprinting barriers to the introduction of apomixis into sexual species.

It has been observed that morphogenesis of embryo is independent of endosperm development, but the normal development endosperm is required for formation of viable seeds. Though the morphogenesis is independent there is evidence though that the genetic control for autonomous endosperm and egg activation may be the same. This interrelationship of embryo and endosperm development has been noted in maize and barley in that defective kernel mutants affect both the embryo and endosperm.

Successful endosperm formation in cereals depends heavily on the specialized cytoplasm of the central cell and requires contributions from maternal and parental genomes. This genomic imprinting in plants is mostly specific to endosperm. The importance of imprinting for embryo and endosperm differs from species to species depending on their roles in the production and acquisition of nutrients. In *Arabidopsis*, gametophytic maternal effect mutation *medea* (*mea*) drastically affects cell proliferation in embryo and endosperm, resulting in seed abortion (Grossniklaus et al. 1998). Genetic and expression studies have shown that *MEA* is regulated by genomic imprinting and expressed both in embryo and endosperm. Proper development of the endosperm in maize is strictly dependent on the presence of maternal and paternal genomes in the ratio of 2M:1P (Lin 1992). Any deviation from this ratio results in failure in endosperm development and causes seed abortion. However in *Arabidopsis* this is not the case, interploidy crosses show endosperm development and produce viable seeds, though there may be difference in the size of the seeds depending on the genotype of the parents.

The imprinting phenomenon may be the reason for high degree of sterility observed in hybrids between sexual and apomictic genera. Thus, genomic imprinting barriers should be given due consideration in efforts to introduce apomixis into sexual species. In gametophytic apomixis the endosperm has 4M:1P ratio still the apomict produces viable seeds, suggesting (1) that the constraints for imprinting are relaxed in apospecies or (2) that the mechanisms of fertilization have been modified. Apomicts show a relaxed requirement for imprinting as the endosperm of different species show different ploidy levels. Apomictic species may be evolutionarily derived from predisposed genera that had relaxed imprinting requirements or, alternatively, evolved specific adaptations of the fertilization mechanism that maintained the imprinting requirements. Such predispositions and adaptations are not thought to exist in most sexual species and imprinting may pose a serious problem to the introduction of apomixis into sexual crop plants.

Screening

A screen for mutants that display apomictic traits in a sexual species could directly lead to the identification of key apomictic regulatory components. Several screens for *Arabidopsis* mutants that allow seed development in the absence of fertilization have been developed. These screens take advantage of male sterile mutants and aims to identify second site mutations that pseudo suppress sterility (Grossniklaus; et al 2001).

Engineering of autonomous apomixes in which both embryo and endosperm develop without fertilization might be considered the ideal situation, but this may be

extremely difficult to achieve in cereals (Grossniklaus; et al 2001). Screens for mutants displaying pseudogamous apomixis in *Arabidopsis* have been attempted. The behavior in which *Arabidopsis* self-fertilizes to reproduce makes these screens very difficult, since they are based on outcrossing and scoring the progeny for exclusive inheritance from the mother plant.

Due to the fact that this method of screening is so hard to use, an alternative to it has been developed that aims at the isolation of pathogenic mutants. It makes use of the *ell* homozygous mutation that produces a large amount of unreduced embryo sacs in *Arabidopsis* (Grossniklaus; et al 2001).

Petunia hybrida is another plant that has been studied extensively for apomictic mutations in conditional male-sterile background. Several transposon genotypes developed from the inbred transposon line W138 were used to generate apomictic mutants. The inbred line W138 contains more than 200 copies of *dTph1*. The *dTph1* transposon system is based on two elements:

1. A non-autonomous transposable element.
2. An autonomous defective element carrying the transposase located on chromosome 1.

The transposable element *dTph1* is 283 bp long with an identical 12 bp sequence in an inverted orientation at each extremity. The *Petunia* transposon system has a high frequency of transposition giving rise to several types of mutations in selfed progeny (CIMMYT et al 1998)

Genetic screens that isolate mutations leading to a lack of reduction are thought to provide useful material to help us better understand the processes plants undergo at the molecular and genetic level (Grossniklaus; et al 2001). Sexual and apomictic reproduction are closely interrelated, thus we use sexual model systems to identify individual components of apomixis. The potential benefits of clonal plant reproduction by apomixis are numerous. Farmers could profit from apomictic hybrid varieties that proliferate their superior characteristics and may make the regular purchase of new hybrid seed obsolete.

Enhancer Detection

To understand the molecular and genetic bases of megasporogenesis and megagametogenesis, scientists are trying to identify genes that are specifically expressed during megasporogenesis and megagametogenesis. The problem is that conventional molecular methods such as differential screening technique cannot be used because of inaccessibility of the developing embryo sac and the relatively small number of cells involved. Therefore a novel technology called enhancer detection has been developed. This technology allows the identification of developmentally regulated genes based on their tissue specificity and pattern of expression.

Enhancer Detection and Gene Trap Systems

Enhancer detection technique was first developed in *Drosophila melanogaster* and depends on a mobile genetic element carrying a reporter gene which is under the control of a weak constitutive promoter (O’Kane and Gehring 1987). This minimal promoter can be activated in all tissues and at all developmental stages. When the

promoter comes under the control of cis-acting regulatory elements like enhancers, then the reporter gene is expressed in a specific temporal and spatial pattern. The pattern of expression of reporter gene reflects the expression of a nearby gene controlled by the same regulatory elements thus, allowing the identification of genes based on their pattern of expression rather than on a mutant phenotype. Similar systems based on T-DNA insertional mutagenesis in *Arabidopsis* or the *Ac/Ds* transposable element system in maize has been developed (Sudershan et al. 1995).

Enhancer detection and gene trap system help in identification of gene(s) acting at different stages of development, for example, a gene that is required for essential steps during both embryo and ovule development can be identified by enhancer detection methods. Enhancer detection has several advantages over classical genetic screens for identification of genes required in gametophytic phase of life cycle. These advantages are:

1. Identification of specific gametophytic genes that act in a cell specific and time dependent manner.
2. Genes required for both microsporogenesis and megasporogenesis can be isolated.
3. Identification of genes expressed in very few or even single cells.
4. This technique allows gene cloning and a detailed analysis of the detected gene through mobilization and the recovery of additional alleles, regional chromosomal rearrangements, and revertant sectors.

Generation of Transposants

Nearly 4,300 lines carrying either randomly inserted enhancer detector or gene trap transposon system were generated to identify genes involved in female gametogenesis. Two screens were set, the first screen was used to identify genes expressed during ovule and megagametophyte development. The second screen was aimed at the identification of genes expressed in individual cell types of the embryo sac including post-fertilization stages. These genetic screens were also used for classical genetic screens to isolate insertional mutants that affect fertility. A PCR-based method was adopted for the rapid isolation and sequencing of genomic regions flanking the *Ds* insertions. Based on the sequence information, the majority of the genes appeared to be involved in gene regulation and signaling processes.

Identification Of Developmentally Regulated Genes and Their Promoters

Very few genes expressed in the megagametophyte have been described to date. Although many *Arabidopsis* promoters have been found to be highly compact enhancers that drive reporter gene expression, these reporter genes could be far away from the site of insertion. Thus making the isolation of the detected gene and its promoter more laborious. The use of enhancer detection technique has led to the identification of transposants expression in individual cells of the megagametophyte. These transposants are located throughout the megagametophyte in areas such as the synergids, the egg cell, and the antipodals.

Some of the genes expressed in individual cell types of the female gametophyte may serve important regulatory functions during sexual reproduction and could be involved in cell specification and differentiation processes. The regulatory regions of these genes will be helpful in the misexpression of candidate genes in particular cell types.

Regulatory genes directing embryo sac, egg apparatus, and egg cell specific expression have been identified. These regions can be used to probe the potential of the egg cell for autonomous activation through misexpression of the candidate genes such as cell cycle control genes and growth regulators. Enhancer detection is the most useful technique for identifying and isolating numerous temporally and spatially regulated promoters for use in modifying the reproductive system in order to develop apomictic lines.

Introduction of apomixis into sexual species

Transferring the apomictic trait to desired plant varieties means fulfilling one of the most cherished dreams of plant breeders, to allow elite cultivars to reproduce without losing hybrid vigor and/or desirable agronomic traits (Rautenberg, 1999). There are many benefits if this dream becomes a reality. Among these are that the apomictic technology could allow developing world farmers access to hybrid technology (Hoisington et al., 1999).

McMeniman and Lubulwa (1997) assessed the development of a 3-phase process proposing to introduce apomixis into rice. They showed that there could be significantly large benefits derived for all rice-growing countries in the world when apomictic rice is produced. These high returns are due to the massive production of rice worldwide and are estimated to bring benefits amounting to \$7 billion to \$8.6 billion worldwide.

Vielle Calsada et al. (1996) also realized the benefits of apomixis in global agriculture given the numerous questions surrounding this trait. Some of the questions that need to be resolved relate to factors influencing sexuality, the genetics of apomixis, and developmental patterns associated with the trait.

The recent review by Grossniclaus (2001) sheds light on the understanding of apomixis and the progress made in introducing it to sexual species. Overall, two general approaches can be followed in introducing the trait:

1. Introgression using classical methods.
2. *De novo* engineering of apomixis through biotechnology.

Introgression using classical methods

An advantage of classical methods is that no prior knowledge of the molecular aspects or of the genes involved is needed. The introduction of apomixis may be done by any of the following three strategies:

1. *Introgression of apomixis from an apomictic wild relative* – Wide crosses can be performed to introduce the apomictic trait from a wild species to the cultivated species. This method was followed by ARS-USDA staff and collaborating scientists where they transferred the trait from eastern gamagrass (*Tripsacum dactyloides*) to corn, thereby producing the first apomictic corn in 1998 (Becker, 1998).

2. *Generation of apomixis through hybridization of sexual progenitors* – This strategy is based on the genome collision theory for apomixis evolution, which assumes that an apomictic hybrid can be obtained by crossing two reproductively divergent ecotypes
3. *Combining reproductive mutants showing some aspects of apomixis* – Crosses between reproductive mutants that exhibit non-recurrent forms may be done to produce an apomictic line.

De novo engineering of apomixis through biotechnology

In theory, it is possible to introduce apomixis to many sexual species using conventional breeding approaches, it is more efficiently done using biotechnology or by the combination of the two approaches. Biotechnology also is the only resort for plants that have no apomictic relative species (Spillane, 2001).

The engineering of apomixis may be done at both gametophytic and sporophytic levels requiring several assumptions and concerted effort in three main areas:

1. Identification and characterization of candidate genes.
2. Isolation of promoters to allow precise control of the genes at both spatial and temporal levels.
3. Development of technologies to introduce and control transgenes and/or perform targeted mutations.

Perotti et al. (2001) reported their study on the collinearity of maize and *Tripsacum* genomes. They assume that genes responsible for apomixis in *Tripsacum* can also be found in maize since both species likely share a common set of genes. In addition, they are using transposon-tagging techniques to characterize the genomic segment that carries apomixis.

Another ongoing study is by Peggy Ozias-Akins at University of Georgia that is looking at pearl millet for apomixis (HPJ, 2002). She hopes to get to the root of apomixis by investigating the genetic makeup of a close wild relative of pearl millet. Pearl millet is grown mainly for cattle feed, but the grain is used in human diets in some parts of the world. Although pearl millet doesn't have the apomixis trait itself, its wild cousin () does. In order to assist the conventional transfer of apomixis into pearl millet, as well as enhance the potential for introduction of this trait across taxonomic boundaries through gene transfer, Dr. Ozias-Akins has an ongoing molecular marker program to tag and clone the gene(s) for apomixis.

Other approaches in engineering apomixis are investigating genes responsible for meiosis, cell wall formation and cell cycle. Regulating genes responsible for a particular step in these processes may influence reproductive patterns. A similar method of hormonal control could be analyzed, as well as gene inactivation and disruption.

Field-level Regulation of apomictic traits

Given that apomixis has been successfully introduced into sexual crop species, the next problem would be controlling its expression especially in breeding programs. If apomixis is the default condition, then it would be impossible to make further improvements to the crop and it could threaten the species genetic diversity. Furthermore, an apomictic trait could add an additional reproductive advantage to the transformed plant thus the potential risks should be considered when this escape to the wild (Van Dijk and Van Damme, 2000). Thus, it would be ideal to have a method of controlling the apomixis behavior at some point during a seed production scheme or a breeding program.

Several inducible control systems offer solutions to this that may allow repression or induction of genes whenever apomixis is required. Examples of systems that have been developed are:

1. Tetracycline receptor system.
2. Copper inducible system.
3. Glucocorticoid receptor system.
4. Glucuronide repressor system.
5. Ethanol inducible system (Salter et al., 1998).

However, many of these regulatory systems are expensive and environmentally unfriendly. Only the glucuronide and ethanol systems are currently being studied for field applications.

Conclusion

Molecular and genetic aspects which lead to apomixis have been discussed and reviewed. Key events result to altered regulation of female gametophyte development and subsequent embryo and endosperm development. It was mentioned that apomixis could be observed only in a very small fraction of the total number of genera of flowering plants.

At present, there have been no full apomicts recovered from sexual species. Mutants displaying the trait and plants exhibiting deviations from the normal sexual reproduction, however, have been documented and studied. Mutant screening and alternative methods, like enhancer detection, have directly led to the identification of key apomictic regulatory components and many candidate genes.

Progress in the study of apomixis during the past years has been tremendous. As realized in 1997, conventional methods led to the development of an apomictic corn. Several other species are currently on the development pipeline. The dream of transferring the apomictic trait to desired plant varieties have been partly satisfied as progress are being made in both introgression using classical methods and the faster method of *de novo* engineering through biotechnology.

The primary issue needing to be resolved is how to deploy the technology in actual field conditions. Even though certain apomixis genes are easily transferred to sexual plants and can be regulated successfully in laboratory conditions, the mechanism of controlling their expressions in the field are not yet fully clear. Other issues relate to the varying difficulties in engineering some types of apomixis than others. Nonetheless,

progress is being made and the release of apomictic transgenics looms on the horizon. Finally, it is viewed that this technology can be fully harnessed if issues relating to intellectual property rights, patenting, and licensing be set aside and the technology made available to the public and private sectors worldwide.

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