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Update on Plant Reproduction

# Apomixis: Molecular Strategies for the Generation of Genetically Identical Seeds without Fertilization[1]

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Sexual reproduction in flowering plants (angiosperms) generates genetically diverse progeny because of the recombination and independent assortment of genes derived from both parents. For commercial agriculture, plants have been selected from the plethora of evolved flowering plant species, and wild species continue to be used as sources of genes in breeding and molecular crop improvement programs. Although we seek and value the diversity in the plant kingdom for use in agricultural breeding programs, once a suitable plant is bred, modern agriculture is dependent on uniformity of seed and vegetable and fruit quality. This demand for product consistency in plantations of often nongenetically uniform populations is clearly at odds with the sexual reproductive mechanisms that have evolved to maximize genetic diversity.

Our reliance on sexual reproduction in agricultural systems also presents vulnerabilities associated with fertilization mechanisms. If agricultural production is pollination dependent, anthers can be sensitive to environmental conditions such as temperature and crop sprays that may inhibit either pollen formation or pollination, thus preventing development of fruit and seed. Fertilization is problematic if flowers are self-incompatible or if plants have single-sex flowers, since there is a 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 fanciful in plants. Some angiosperms commonly reproduce in this manner by a process called apomixis. The purpose of this review is to briefly describe apomictic development, argue for the use of apomixis in agricultural production, consider the genetic basis of apomixis, and 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.

## APOMIXIS: AN ALTERNATIVE METHOD OF SEED PRODUCTION IN PLANTS

Apomixis occurs throughout the plant kingdom, from algae to angiosperms (Asker and Jerling, 1992). 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 (Asker and Jerling, 1992). Both monocotyledonous and dicotyledonous angiosperm taxa are known to include apomictic species. The most well-represented families are the Gramineae, Compositae, and Rosaceae (Richards, 1986), although this bias may be more reflective of research effort than natural abundance.

The developmental details of various apomictic processes have been discussed in recent reviews (Asker and Jerling, 1992; Koltunow, 1993; Naumova, 1993). Apomictic processes occur in the ovule, resulting in progeny that are genetically exact copies of the female plant because fertilization is unnecessary to produce an apomictic embryo. The apomictic embryo is formed in the ovule via two fundamentally different pathways, sporophytic or gametophytic, which define the origin of the apomictic embryo (Gustafsson, 1946; Asker and Jerling, 1992; Koltunow, 1993). Figure 1 shows that 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 (Fig. 1), two mechanisms are generally recognized, 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 (Fig. 1), the embryo sac originates from megaspore mother cells either directly by mitosis and/or after interrupted meiosis. In apospory (Fig. 1), the embryo

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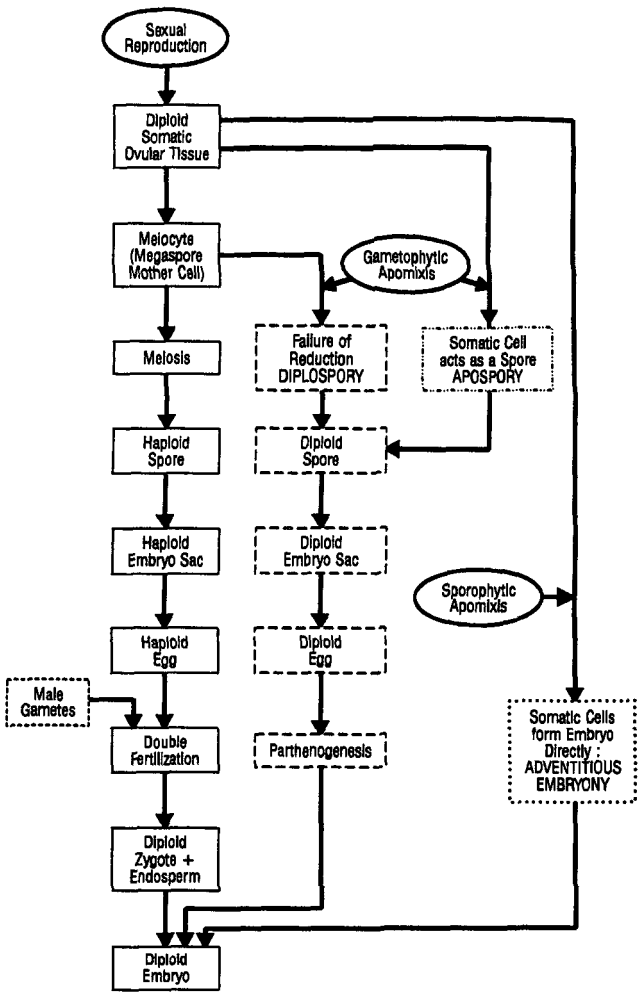


**Figure 1.** Comparison of sexual and apomictic pathways in angiosperm ovules. The figure shows differences between the two gametophytic pathways, apospory and diplospory, and the sporophytic pathway of adventitious embryony, all compared with the sequential events of sexual reproduction.

sac originates from nucellar cells (Gustafsson, 1946; Koltunow, 1993). In both gametophytic mechanisms, the resulting nuclei forming the embryo sac are of the same ploidy as those found in the female parent because the reduction division cycle of meiosis does not occur. The embryo arises autonomously from one of the cells in the embryo sac.

Irrespective of whether sporophytic or gametophytic apomixis occurs, generation of a fertile seed can still be dependent on fertilization for the formation of endosperm. Pseudogamous apomicts are those in which seed formation depends on the fertilization of the polar nuclei for endosperm development. By contrast, autonomous apomicts develop an endosperm independent of fertilization of the polar nuclei by a process called autonomous endospermy.

Apomixis and sexual reproduction are not necessarily mutually exclusive events. In plants termed obligate apomicts, sexual reproduction is essentially excluded, since all of the seeds harvested have the genotype of the female parent. In facultative apomicts, both zygotic and clonal seeds can be harvested from the same plant. Therefore, in facultative apomicts occurrence of the trait is not absolute and both sexual and apomictic processes can coexist, whereas obligate apomixis may represent a more complete suppression of sexuality (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. The male gametophyte makes no contribution to the genetic makeup of the embryo, which ensures that the genotype is fixed and maternal in origin. 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). Surprisingly, a viable seed is produced.

## THE BENEFITS OF APOMIXIS TO AGRICULTURE

Hanna and Bashaw (1987) recently described how apomixis 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 agricultural production. They are the first-generation progeny ($F_1$) between two genetically different plants or inbred lines. $F_1$ hybrid plants are heterozygous, normally uniform within the group, and may exhibit hybrid vigor, which is a sought-after feature. Hybrid cultivars cannot be used as seed sources in the next generation ($F_2$) because this generation would be extremely variable as a result of genetic segregation. Hybrid vigor is also reduced in the $F_2$ 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 vigor and the development of true-breeding hybrids in a particular breeding program. Seed could be produced for many generations without loss of vigor or genotype alteration. Hybrid seed production would be simplified because line isolation would not be necessary to produce $F_1$ 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, apomixis 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

rights and patents should protect the owners of new plant lines, regardless of their method of production. Furthermore, it is likely that the purchase of seed from a specialty producer would continue in large-scale production concerns to ensure quality. An understanding of the action of apomixis at the molecular level may eventually make it possible to control apomixis in commercial, hybrid seed production so that apomictic ability will no longer be maintained after a single generation, allowing the commercial protection of elite hybrid lines.

New apomictic genotypes could be produced by conventional hybridization of sexual and apomictic plants using the apomict as the pollen parent because apomicts usually produce functional male gametes. Breeding of new varieties would be accelerated: progeny tests for genetic stability would not be needed because the plants would breed true and would be ready for immediate performance testing. Thus, crops could be more easily tailored to match high performance to a particular climatic region.

The use of apomixis also holds great promise as an essential tool for plant improvement and sustainable agricultural production in developing countries with limited land mass for food production. For example, in many developing countries rice is the major food source, and many farmers tending small paddies are responsible for production. Rice is naturally self-fertile and most varieties are maintained as inbred lines. It has been demonstrated, however, that yield improvements of 20% are possible using hybrid rice varieties. Hybrid seed currently must be purchased by farmers each season, and at a cost 5 to 10 times higher than that of conventional seed, the purchase of hybrid seed is prohibitive to most farmers (David, 1991). Apomixis in rice would enable farmers in developing countries to benefit from continued hybrid vigor by being able to use seed from the previous harvest to plant their next crop. This is in contrast to the situation in developed countries, where purchase of seed is likely to remain the norm for the limited percentage of the population involved in agricultural production because seed purchase is only a relatively small component of the total production cost.

## THE GENETIC BASIS FOR APOMIXIS

In each of the apomictic pathways depicted in Figure 1, it has been established from studies of hybrids between sexual plants and their apomictic derivatives (apomict as the pollen parent) that apomixis is under genetic control. In most of the plants studied to date, only a single, dominant gene locus is necessary to elicit each of the apomictic pathways shown in Figure 1 (Koltunow, 1993).

Although apomixis is scattered throughout the plant kingdom, few important agricultural crops possess this trait. Adventitious embryony is common in *Citrus*, orchids, mangoes, and mangosteen (Naumova, 1993). Aposporyis found in Compositae (Richards, 1986). It is also prevalent in Gramineae, particularly in the forage grass crops and wild relatives of grain crop species, for example, *Pennisetum squamulatum*, which is related to pearl millet (*Pennisetum glaucum*; Bashaw and Hanna, 1990). Diplospory is found in Compositae such as *Taraxacum* (Richards, 1986). Diplospory is also found in Gramineae such as *Elymus* and *Tripsacum*, which are wild relatives of the grain crops wheat and maize, respectively (Bashaw and Hanna, 1990).

Therefore, most research to date has centered on introgressing the trait of apomixis into agricultural crops of importance, such as wheat and maize, from wild, often very distant relatives, by traditional breeding. Apomixis has been successfully transferred to pearl millet (Dujardin and Hanna, 1989). However, the breeding approach is slow and often impeded if apomixis does not occur in wild relatives of the agricultural crop of interest and/or if breeding barriers prevent introgression from unrelated species. Furthermore, the choice of the type of apomictic mechanism that can be introduced is limited by what is conveniently available in the closest apomictic relative, and it may not be the mechanism most optimal for agricultural purposes. These problems could be surmounted if molecular knowledge of the genes involved in initiating and controlling apomixis was obtained, because the genes could be transferred directly to the crop of interest by molecular transformation. The success of this molecular approach is also dependent on the existence of a gene introduction and plant regeneration system for the particular agricultural crop.

Although the origins of the cell that becomes an apomictic embryo are different from those of the zygotic embryo, the apomictic embryo is formed and nurtured by processes that mirror normal, postmeiotic embryo sac formation (Chaudhury and Peacock, 1993). The subsequent development of the embryo, endosperm, and seed are also comparable to the processes that occur in sexual reproduction. In facultative apomicts there is a coexistence of sexual and apomictic reproduction in an individual ovary and often within a single ovule. Any hypothesis concerning the nature of an apomictic gene product and its mode of action needs to satisfactorily accommodate all of these observations. It is unlikely that the apomictic locus responsible for all of these events involves a distinctly new pathway that includes new genes for embryo sac formation and embryogenesis. Apomixis and sexuality are not mutually exclusive events, because in facultative apomicts the processes coexist (Koltunow, 1993). One possibility is that apomictic reproduction is caused by the erroneous expression, in both developmental position and time, of a gene that normally functions to initiate cascades of gene actions at different times during the course of sexual events in the ovule (Peacock, 1992; Koltunow, 1993). The apomictic phenotype could be due to a mutated allele of such a gene.

## *HIERACIUM*, AN APOMICTIC MODEL SYSTEM

We currently know almost nothing about apomictic genes or apomictic gene action. One approach toward the isolation of apomictic genes would be to choose an apomictic mode of reproduction that would have the best potential for agricultural systems, find a plant with that apomictic mechanism that also possesses suitable features for the molecular study of apomixis, and then develop the plant as an experimental system. In this plant species, the aim would be to study apomixis at the developmental, cell

biological, and molecular levels in comparison with sexual reproduction in a purely sexual sibling.

Selection of a known apomict and the development of experimental methods for its use as a model system provide the advantage of studying an operative, intact system that utilizes a mechanism of apomixis chosen for its amenability. As with all model systems, the plant should be easily cultivated both in vivo and in vitro, with a small stature, short generation time, and abundant seed set to facilitate rapid turnover of experimental populations. Both sexual and apomictic biotypes need to be available. The apomictic mechanism should employ autonomous endospermy to avoid difficulties associated with pseudogamy. Male meiosis and pollen formation must be functional in the apomictic biotype, since the segregation and transfer to the sexual recipient of alleles associated with apomixis is typically possible only through pollen. Finally, apomixis needs to be easily assessed and quantified in the plant in a manner that preferably avoids the usual, tedious, histological method of sectioning. Rapid and accurate quantification of apomixis is necessary during crossing experiments between sexual and apomictic plants to facilitate the evaluation of allelic differences and any additive and/or epistatic influences of modifier loci (Bicknell, 1994a).

To facilitate a molecular study it is important that the model apomictic plant can be genetically transformed, permitting the introduction of marker genes, mutagenic sequences (e.g. T-DNA tags and transposons), and the reintroduction of putative control sequences. It would also be preferable for the model plant to have a small genome and ideally be already characterized with respect to morphological and molecular markers (Bicknell, 1994a).

What apomictic mechanism would be most useful in agriculture? A decision needs to be made between sporophytic and gametophytic apomixis and whether the mechanism should be obligate or facultative. An obligate apomict may not be desirable in agricultural systems, since an inability to switch back to sexual reproduction would reduce options for breeding new varieties from that plant. The obligate apomict may have a short-term fitness advantage, but it is essentially doomed in an evolutionary sense. The facultative apomict, however, can combine short-term fitness of a particular genotype with a long-term future by switching between asexual and sexual modes of reproduction (Peacock, 1993). It is important, however, that the proportion of apomictic progeny in the facultative apomict be sufficiently high for agricultural purposes.

Sporophytic apomixis is not a very useful mechanism because it is coupled to pseudogamy and is therefore dependent on the presence of a sexual embryo sac in the same ovule to develop an endosperm to nourish the clonal embryo (Koltunow, 1993). Of the facultative, gametophytic apomicts possessing autonomous endospermy, the choice between diplospory and apospory is difficult. In an ovule undergoing diplospory, the fate of the megaspore mother cell is altered onto an apomictic pathway and it is not technically possible for sexuality and apomixis to coexist in such an ovule. Thus, that ovule can only be sexual or apomictic in developmental potential. Facultativeness in a diplosporous apomict is determined by the proportion of ovules in the flowers of the plant that have the capacity to undergo either sexual or apomictic development. In ovules undergoing apospory, sexual and apomictic processes are able to coexist within a given ovule, and the proportion of sexual or apomictic seed depends on the timing of sexual and apomictic processes within an ovule in addition to the proportion of ovules in the flowers of a particular plant that are able to initiate apomictic processes (Koltunow, 1993). Whether diplospory or apospory is more beneficial for agricultural production remains to be determined.

Experimentally, however, apospory is more attractive because it is easier to detect histologically, because aposporous processes are usually initiated after the initiation of sexual reproduction. Given that both sexual and apomictic processes can coexist within a given ovule, experiments can be conducted that should provide an indication about the degree of relatedness between the genes being expressed during sexual and apomictic reproduction (Koltunow, 1993). It should be possible to monitor changes in, for example, mRNA populations between both processes as long as there is a sufficient time interval between the initiation of the two pathways. Thus, even if genes of a similar sequence are involved in both processes, it should be possible to observe whether they have the same or different patterns of expression in two different developmental pathways. Apospory also has the experimental advantage that the selective inactivation of either the sexual or apomictic pathway through mutation is likely to lead to the exclusive expression of the other.

Several model systems for apomixis have been previously proposed. Most have been monocotyledonous species related to grain crops, including members of the following genera: *Pennisetum, Brachiaria, Tripsacum, Elymus,* and *Panicum* (Bashaw and Hanna, 1990). Monocotyledonous species are difficult to transform, which imposes limitations on the approaches that can be taken to identify and isolate the genetic elements involved in apomixis. These monocotyledonous, apomictic species often have long generation times and are difficult to emasculate, which is important for the quantitation of apomixis. Aposporous, dicotyledonous species that have been proposed as model systems include members of the genus *Potentilla* and also *Ranunculus auricomus;* however, these species are pseudogamous (Nogler, 1984).

The taxon that appears to be most suited for use as a model system for a molecular study of apomixis is *Hieracium,* subgenus *Pilosella,* a compilation of over 60 apomictic biotypes native to Eurasia and North America (Bicknell, 1994a). Figure 2 shows *Hieracium pilosella,* which like other *Hieracium* species is a small, herbaceous perennial, easily propagated and maintained in the greenhouse. *Hieracium* species are typically LD plants, flowering in response to an extended photoperiod (Bergstrom, 1969; Yeung and Peterson 1971). Daylength-extension lighting can be used to encourage flowering throughout the year. Seed is set within 3 to 4 months of germination, which allows three to four generations per year (Bicknell, 1994a).

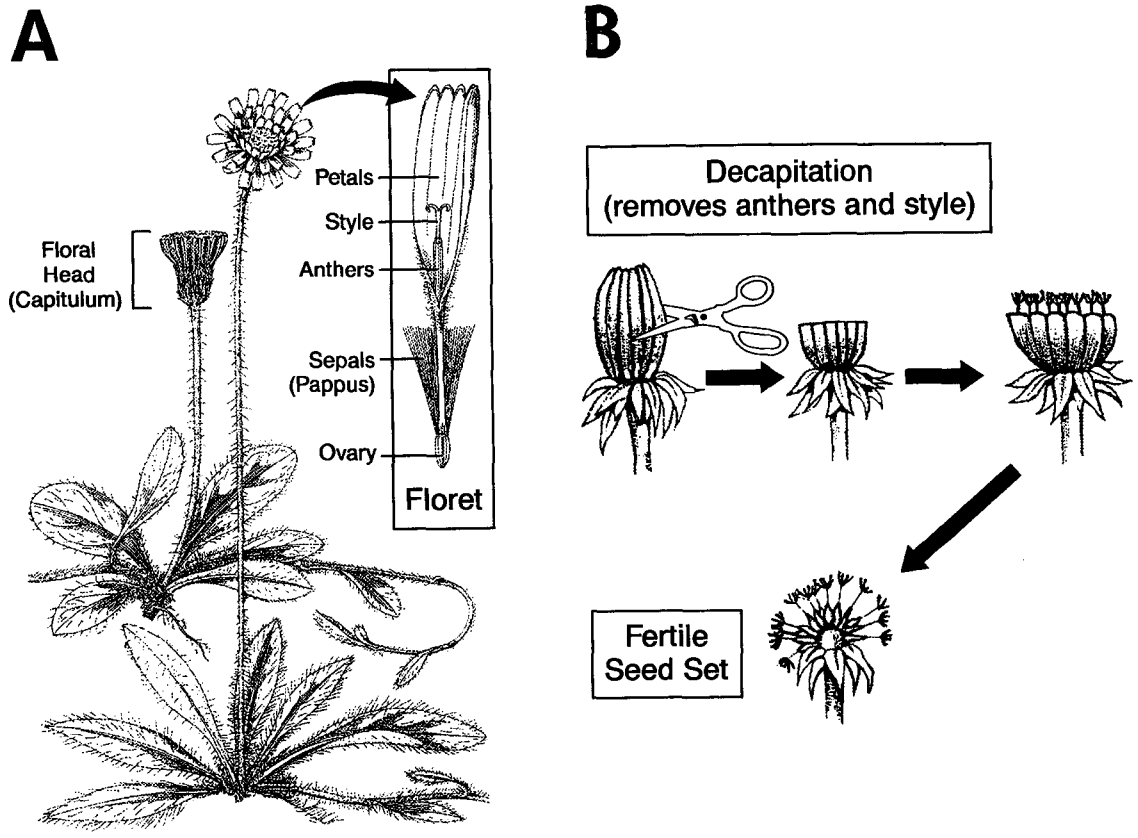


**Figure 2.** *H. pilosella*, an aposporous apomictic plant for the isolation and study of apomictic genes. A, *H. pilosella* and details of floral morphology. (Figure is modified from Ross-Craig, 1960.) B, The degree of apomixis in *H. pilosella* is easily assayed by decapitation of the unopened floral bud, which removes the stigma and the anthers. Fertile apomictic seed that is set can be counted. (Figure is modified from Richards, 1986.)

In *Hieracium* seed develops by facultative apospory coupled with autonomous endospermy (Richards, 1986); therefore, pollination is not required for the formation of clonal seed. The capitulum of *Hieracium* is a compound inflorescence containing 60 to 120 individual florets (Fig. 2). Decapitation of the capitulum at an immature bud stage removes both anthers and stigmas, which prevents sexual seed formation but not clonal seed set, and thus apomixis can be scored by the percentage of seed set after decapitation (Fig. 2).

*Hieracium* species are polyploid, and reported examples range from triploid to octaploid (Tutin et al., 1976). Apomicts are typically polyploid, particularly gametophytic apomicts. Although polyploidy is not thought to be essential for initiating apomixis (Asker and Jerling, 1992), it is possible that high ploidy levels may contribute modifiers or other genetic factors that could affect the level of apomixis and its susceptibility to environmental influence (Nogler, 1994). The range of ploidy in *Hieracium* offers an opportunity to produce populations of plants segregating for apomixis and sexuality and possessing varying levels of ploidy to address more stringently the relationship of ploidy to the degree of apomixis.

Mendel appears to be the first to have studied the genetics of *Hieracium*, ironically to help corroborate the laws of inheritance he had formulated from his work in *Pisum* (reviewed by Nogler, 1994). *Hieracium* did not conform to Mendel's laws, which stimulated intense correspondence between Mendel and Carl Nägeli, a *Hieracium* specialist (Nogler, 1994). Ostenfeld (1906) first recorded apomixis in *Hieracium*, and the embryology of various members of the genus has been subsequently documented by a number of researchers (reviewed by Gustafsson, 1946). Gadella (1991) recently found that apospory in *H. pilosella* is controlled by a dominant allele at a single locus.

Although *Hieracium* has been studied for more than 100 years, very little information is available on its experimental manipulation in the laboratory. The apparent suitability of this taxon for use as a model system stimulated an effort to develop methods that would aid in the molecular analysis of apospory. A range of tissue-culture techniques has now been described, including methods for shoot regeneration from leaf tissue (Bicknell, 1994b) and the recovery of genotypes reduced in their ploidy levels from anther culture. An efficient genetic transformation system has been developed (Bicknell and Borst, 1994) in which, depending on the variety of *Agrobacterium* strain and plasmid used, efficiencies ranged from 5 to 40% of explants yielding a transformed shoot. Chimeric genes conferring resistance to spectinomycin, kanamycin, hygromycin, and 5-fluorocytosine sensitivity and conferring GUS activity have been introduced, and their activity and stable inheritance have

been demonstrated (Bicknell and Borst, 1994). Transposable elements from maize have been introduced by plant transformation and have been demonstrated to move in *Hieracium*. The functionality of the maize transposon system in *Hieracium* offers the potential for its use in a mutagenesis screen to "turn off" apomictic genes and to search for completely sexual progeny (Bicknell, 1994a).

Hybridization studies between sexual and apomictic *Hieracium* species have led to the selection of sexual and apomictic siblings for use in comparative molecular studies. Careful selection of mRNA populations at different developmental time points coupled with comparative screening will determine whether similar genes are being turned on in similar patterns during sexual and apomictic reproduction. The comparative studies will also supply developmental and cell biological information to complement the transposon mutagenesis screens in *Hieracium*. Functionality of any candidate apomictic gene located by either mutagenesis or PCR subtraction methods can be tested by introducing the gene into the sexual *Hieracium* line by plant transformation.

## ARABIDOPSIS: CONVERSION OF SEXUAL PLANTS TO APOMICTIC PLANTS BY MUTAGENESIS?

If apomixis is controlled by a dominant locus, and if the apomictic phenotype is due, as hypothesized above, to a mutated allele of a gene normally involved in sexual reproduction, then another strategy toward the isolation of an apomictic gene could be to mutagenize a sexually reproducing plant in an attempt to convert it to an apomictic mode of reproduction. Mutation of a sexual plant might, for example, result in the inactivation of a transcriptional repressor that normally suppresses an apomictic pathway, or it might result in a plant with a transcription factor with a novel specificity, capable of inducing an apomictic reproductive pathway (Chaudhury and Peacock, 1993).

Attempting to mutagenize a sexually reproducing plant into apomixis is not an easy task because the sexual reproductive system can mask the formation of apomictic progeny, making it difficult to determine whether a sexual or an apomictic pathway was used during seed development. It is necessary to abolish sexual seed formation before mutagenesis to induce apomixis is carried out.

*Arabidopsis thaliana* is a convenient, sexually reproducing plant in which to construct a screen for apomictic mutants. It is a proven experimental system for mutagenesis and the isolation of genes involved in various plant processes. It is small in size and has a rapid life cycle, which means that many plants can be screened after mutagenesis. The small genome size, limited repetitive DNA (Meyerowitz, 1987), and worldwide collaboration for accessibility to mapped molecular markers, mapping lines, and sequence information means that once a gene is identified it can be isolated relatively easily. Apomixis is not known in Arabidopsis, although diplosporous apomixis has been reported in the crucifer *Arabis holboellii* (Asker and Jerling, 1992).

One way to dispense with the potential for sexual reproduction in Arabidopsis, which is a self-fertilizing plant, is to create a line that is male sterile and therefore unable to self-pollinate to produce sexually derived seeds. When such feminized plants are mutagenized, any seed production would be indicative of a nonsexual or apomictic mode of reproduction (Chaudhury and Peacock, 1993). Arabidopsis mutants such as *apetala* (*ap3*) and *pistillata* (*pi*) have nonfunctional male reproductive organs, but female fertility is unimpaired (reviewed by Coen and Meyerowitz, 1989). Such Arabidopsis mutants are suitable genetic backgrounds for mutagenesis and screening for apomictic mutants.

Mutagenesis of sexual Arabidopsis to an apomictic plant, if it is at all possible, would be an extremely rare event. The screen should therefore be designed to enable visual detection of an apomictic plant and to avoid histological methodologies until putative apomictic plants are identified. Arabidopsis is very attractive for this purpose because seed development is coupled to pistil elongation. If seed formation does not begin, the pistil does not elongate significantly from its length at flower maturity and remains as a short stub on the inflorescence. For example, male-sterile *pistillata* mutants develop only short siliques. To maximize detection of an apomictic plant the screen should be conducted to detect mutations in both dominant and recessive genes. There is also a possibility that mutagenesis may result in an Arabidopsis plant capable of initiating embryogenesis but incapable of completing development to form a viable seed because of the absence of endosperm production (i.e. a pseudogamous apomict). To identify potential pseudogamous plants, the mutagenesis screen also needs to incorporate pollination postmutagenesis.

Figure 3 shows a simple visual screen, currently in progress, that has been designed to detect induced apomictic mutants in Arabidopsis, assuming that apomixis is controlled by a single gene (Chaudhury and Peacock, 1993). The expected phenotype of a bona fide apomictic plant in this screen is one in which silique elongation occurs in stamenless flowers and fertile seeds are produced.

The dominant apomictic locus of naturally occurring apomictic plants may contain a number of different, tightly linked genes that separately induce different components of the apomictic pathway (i.e. avoidance of meiosis, autonomous embryo and endosperm formation). If this is the case, then mutagenesis of a sexual plant such as Arabidopsis may not easily produce an apomictic plant capable of setting viable seed in the absence of pollination. However, it may be possible to detect mutants exhibiting individual components of apomixis in which meiosis and female gametophyte development were uncoupled and double fertilization was uncoupled from embryo and endosperm development. In the screen described in Figure 3, such mutants may exhibit partially or fully expanded siliques, perhaps containing aborted seeds that may or may not contain embryos and/or endosperm.

## CONCLUSIONS AND PERSPECTIVE

Most studies of apomixis have focused on describing the cellular mechanisms employed, or on the ecological implications of the trait for different species. The genetic studies of apomixis referred to here show that molecular ap-

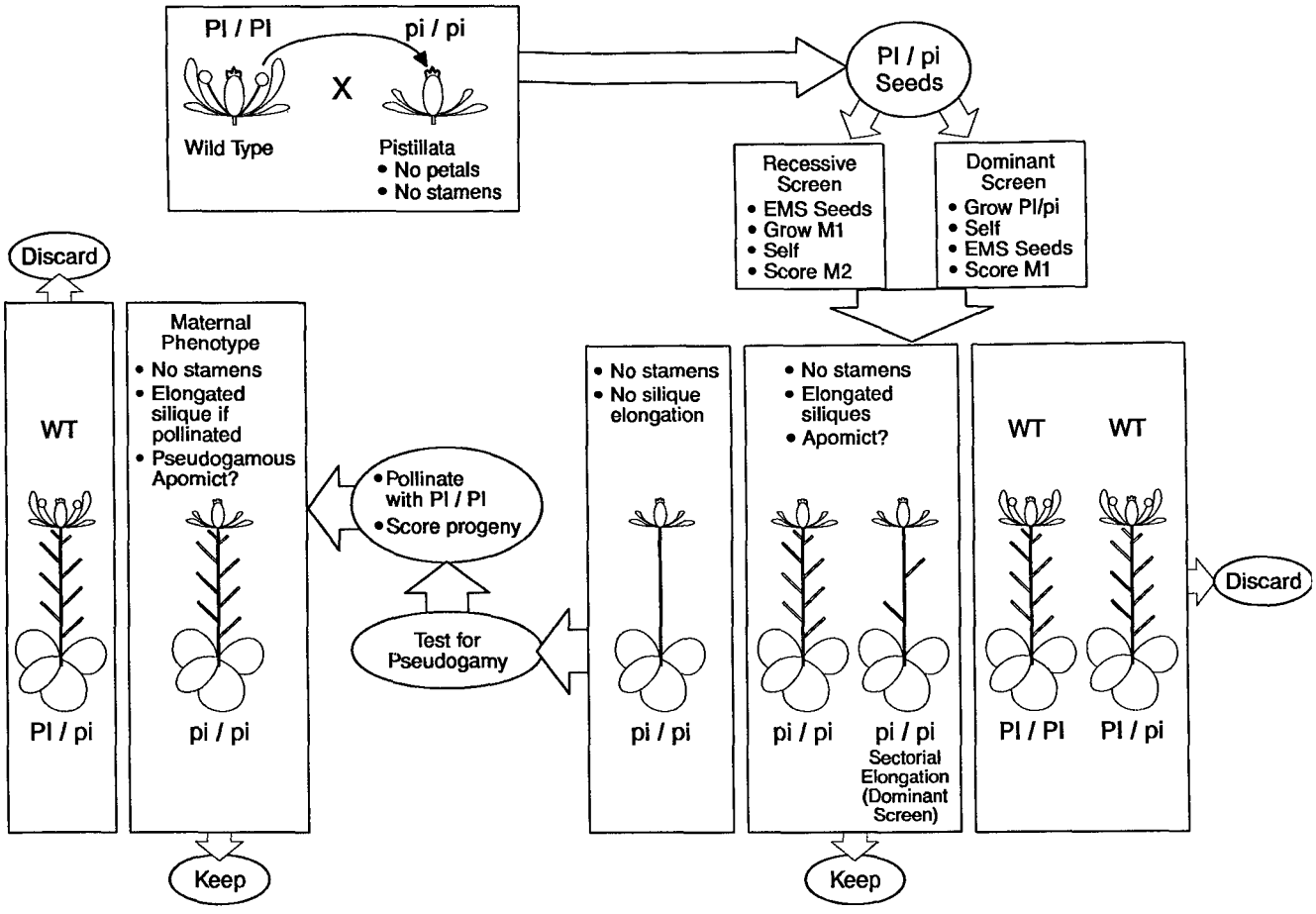


**Figure 3.** A visual mutagenesis screen in Arabidopsis to detect the mutation of a sexual plant into an apomict. In this drawing, Arabidopsis plants are depicted in an abbreviated form for simplicity, where the floral phenotype is shown as a single terminal flower (not normal in Arabidopsis) and elongated siliques carrying seeds are shown as short branches on the inflorescence meristem. Nonelongating siliques lacking developing seeds are not depicted. The strategy of the screen can be followed by tracing the path of the arrows from the top left-hand box. In this screen, the recessive *pistillata* mutation (pi/pi), which displays male-sterile flowers and lacks petals in homozygous plants, is used as the pollen acceptor line in a cross with wild-type (PI/PI) plants to produce PI/pi heterozygous seeds. These seeds form the genetic background for subsequent mutagenesis. To detect mutations in recessive apomictic genes (Recessive Screen), several thousand PI/pi seeds are mutagenized with ethyl methanesulfonate (EMS) and the $M_1$ plants are selfed. The $M_2$ plants with pistillata (male-sterile) flowers are screened for the apomictic phenotype where elongated siliques form in the absence of self-pollination. In the recessive screen elongated siliques carrying developing seeds would be seen all over the apomictic plant. For detection of dominant apomictic genes (Dominant Screen), the PI/pi seeds are grown up and selfed, progeny seeds are collected and mutagenized with EMS, and the $M_1$ plants are grown and scored directly for male-sterile flowers, elongated silique phenotype. In contrast to the recessive screen, a dominant mutation in the $M_1$ plant may be seen as an elongation of siliques in only a portion or sector of the plant, as shown in the diagram and marked "Sectorial Elongation (Dominant Screen)." In both dominant and recessive screens, if the mutation results in a pseudogamous apomict, then the expected phenotype would be a plant containing male-sterile *pistillata* flowers that lack siliques, a phenotype indistinguishable from the *pistillata* plant. To detect pseudogamous apomicts among these plants, they need to be pollinated with wild-type pollen (PI/PI) to determine whether the resultant progeny are maternal or sexually derived. After the pi/pi male-sterile plants are pollinated with PI/PI pollen, most of the progeny will be sexual heterozygotes (PI/pi), although the rare pseudogamous apomict will retain the maternal stamenless phenotype (pi/pi) and will be capable of producing elongated siliques if the flower is pollinated.

proaches toward isolating the genes controlling the process are feasible. Such studies are now in progress. Introgression programs that introduce the trait into agriculturally important crops by conventional breeding will continue in parallel, but direct isolation of genes conferring apomixis development would greatly facilitate the transfer of this trait to a much wider variety of crops and agricultural applications.

Rice production provides a good example of the potential impact of this trait. Considering rice consumption in the context of projected increases in world population, it will be necessary to produce enough rice to meet the needs

of 4.3 billion new rice consumers in the year 2025 (Khush et al., 1994). High-yielding, hybrid rice seed can provide yield increases of up to 25% over conventional rice seed. Apomictic production of hybrid rice seed would provide the benefits of hybrid vigor to producers in developing countries and would offer an economically viable way of delivering increased production to developing economies for improving both agricultural and nutritional performance. In developed countries where agriculture and the dollar are tightly linked, the same benefits of apomixis would streamline productivity, make the breeding of new crops more rapid, economize practices in the hybrid seed industry and in tree crops, and decrease losses in yield that occur due to unfavorable pollination conditions.

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