

Apomixis and the Management of Genetic Diversity

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Introduction:

Apomixis is a form of asexual reproduction in many plant species which results in the formation of seeds genetically identical to the mother plant. The introduction of apomictic reproduction into today's important agronomic crops could offer tremendous benefits to plant breeders and farmers throughout the world. Currently, plant breeders achieve tremendous yields through the production of hybrids derived from the crossing of two highly inbred lines. The generation of these lines is a time consuming process that takes years of crossing and self-pollination. Then, after the inbreds are crossed and the rewards of hybrid vigor have been realized, the process must be started over again. This is because the seeds of the hybrids are highly variable in genetic makeup and any attempt to plant them would result in a generation with a random mix of yields. However, hybrid plants using apomixis as their reproductive mechanism would not be subject to this variability (1). All of their seeds would be genetically identical to the mother plant and the seeds could be planted generation after generation with the same agronomic results. (Figure 1) Furthermore, in crop species which have a stable cytoplasmic-nuclear male sterility system for the production of hybrid seed (maize, sorghum, and pearl millet), apomixis would enable breeders to more easily select unique gene combinations which could then be used to maximize hybrid yield.

With a world population growing at nearly 160 people per minute, it has been estimated that the world will need to produce as much food during the next 50 years as was produced since the beginning of agriculture nearly 10,000 years ago (2). Therefore, the incorporation of apomixis would be a valuable tool enabling plant breeders to produce high yielding hybrid lines more efficiently and maintaining their gains through subsequent generations. Apomixis occurs naturally in approximately 40 plant families and in more than 400 plant species, but none of the major global cash crops reproduces through apomixis at the present time. However, it could be possible to introduce apomixis into these crop lines through the use of genetic engineering. Therefore, scientists around the world are actively pursuing the triggering of apomixis in today's major cultivated crops through gene transfer methods (3).

Still another benefit of apomixis, which would be of great importance to farmers, is the fixation of locally adapted hybrid varieties. All too often, farmers subject to a geographically specific disease or pest do not benefit from a hybrid seed which cannot tolerate their problem. Therefore, selection of apomictically reproducing local hybrid varieties that have already proven their hardiness against local factors would be very valuable to breeders trying to develop region specific hybrid seed.

A major concern with the use of apomixis is the loss of genetic diversity associated with sexual reproduction. In obligate apomictic plants, which can only reproduce through apomixis, the ability to generate genetic variability is at an evolutionary dead end. Their only source of diversity would come in the form of somatic mutations. As a result, a simple change in the environment, a new herbivore, or disease would be devastating to a population incapable of adapting to change. On the other hand, facultative apomixis allows plants to reproduce through both asexually and sexually in various proportions. Therefore, the opportunity for genetically variable progeny is still available. Since access to a wide range of genetic diversity is critical to the success of breeding programs, this is a topic which must be considered when talking about the generation of apomictic crops.

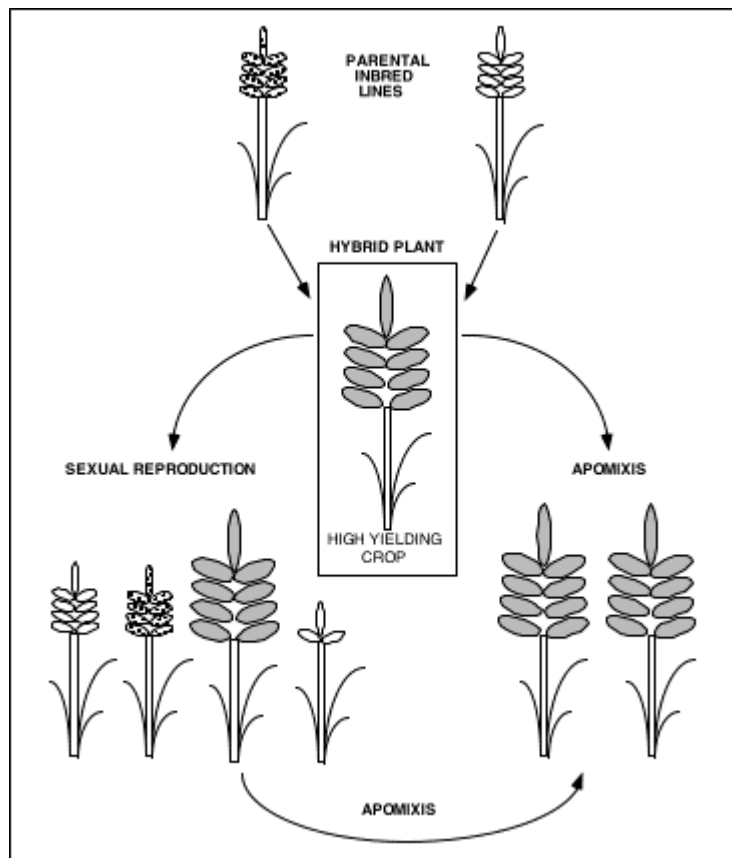


Figure 1: Hybrid plants are more vigorous and higher yielding than their parents. Sexual reproduction creates variability among the progeny which results in lower yields. Apomixis allows for the fixation of hybrid vigor and production of cultivars from one exceptional plant.

Progeny of Apomictic Plants

In most apomictic plants, fertilization of the endosperm is required for continued development while the embryo develops through parthenogenesis (Figure 2). This process is referred to as pseudogamy (4). Therefore, in general, two steps are needed for apomixis to occur: 1) development of an embryo sac without reduction, and 2) parthenogenetic development of the embryo without fertilization. The result is a $2n + 0$ embryo which is a clone of the maternal plant. With this mode of reproduction, generation of higher ploidy levels is favored. The movement towards increased ploidy is the result of fertilization within an unreduced embryo sac. Conversely, changes towards lower ploidy come through meiosis and genetic recombination which occur through sexual reproduction. For the most part, diploid plant forms are sexual and polyploid forms are apomictic.

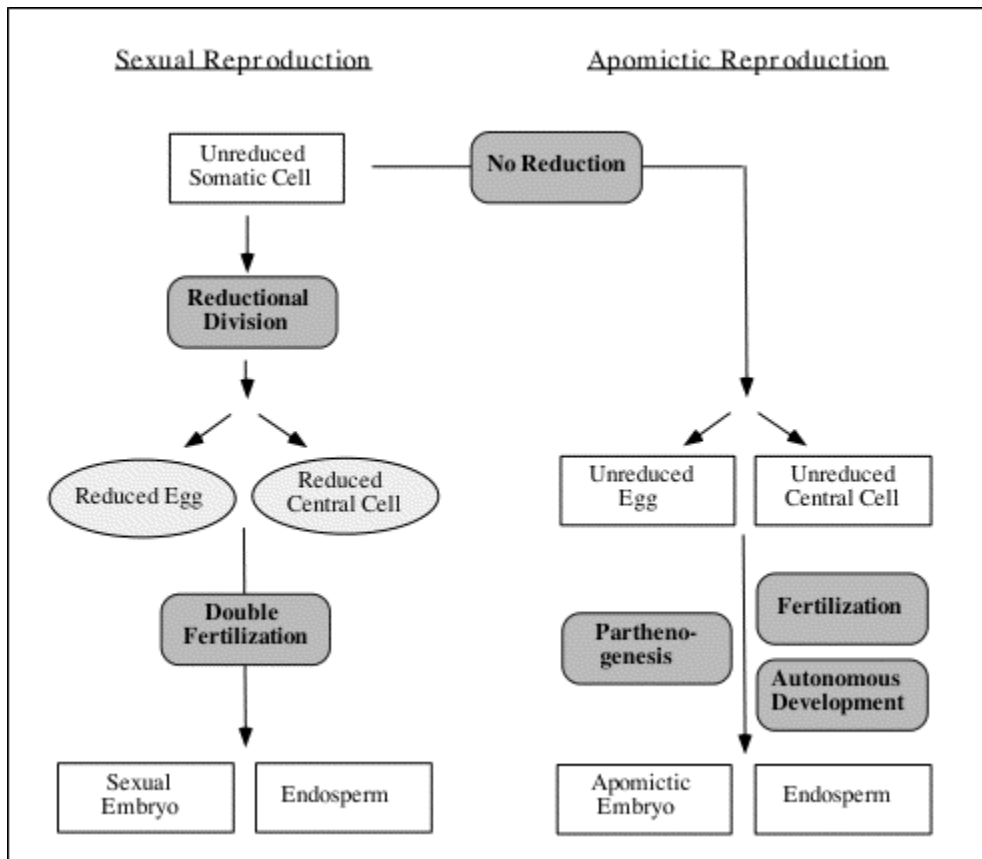


Figure 2: The normal sequence in sexual reproduction is short-circuited so that the next step is initiated before the preceding one is completed.

Cycles and Sexuality

In all agamic complexes, two different pools of ploidy are found. The first is a lower ploidy pool (usually diploid) which reproduces sexually, and the second pool is a higher ploidy level (predominantly tetraploid) which usually produces through apomixis. The lack of apomixis in the lower ploidy pool is believed to be the result of a lack of

expression of this trait at this level or to an absence of transmission through haploid gametes. The bulk of the genetic diversity is present in the sexual diploid pool where genetic recombination is occurring (4).

Gene flow can occur from the diploid pool to the polyploid pool through a few different channels. One channel being the infrequent formation of $2n$ gametes in sexual plants. If these gametes are pollinated by pollen of apomictic tetraploid plants, tetraploid plants will be produced that are still apomictic to some extent. This will allow a burst of genetic diversity to occur in the higher ploidy pool. Another channel is the pollination of unreduced eggs from triploid plants by normal pollen grains of diploid plants. These triploids can arise through crosses between diploid and tetraploid plants. Therefore, there are several avenues for genetic diversity to enter the higher ploidy pool.

Comparison of Genetic Diversity in Three Orchid Species

Researchers conducted a study on the genetic diversity of three orchid species in 2001. (5) The study was intended to assess the genetic variation and its partitioning within and between populations of the three species for formulating conservation management strategies. The species in question are *Zeuxine gracilis*, *Zeuxine strateumatica*, and *Eulophia sinesis*. Each of these species reproduces by a different method.

Z. gracilis is an outcrossing orchid species with restricted distribution found in damp, shaded woods in mountainous areas. *Z. strateumatica* reproduces through apomixis and is located in newly available habitats such as damp open grassland and open slopes. *E. sinesis* is an outcrossing colonizer and is generally found in open fields of construction sites or newly generated grassland.

Previous allozyme studies on the apomictic *Z. strateumatica* found no genetic variation within the species. However, it was thought that this initial allozyme study may not reflect the true amount of variation within the species. Low levels of allozyme polymorphisms also hindered the study of genetic variation within and between populations. Further molecular marker studies covering a larger portion of the genome needed to be conducted to confirm the actual level of variation and to complement the original study.

After weighing the positives and negatives associated with the many types of DNA markers available today, the type of markers chosen to conduct this next study were RAPD (randomly amplified polymorphic DNA) markers. On the negative side, RAPD markers are subject to low reproducibility and marker allele dominance problems. However, for this study the positives were thought to outweigh the negatives. The researchers chose RAPDs because they can provide much higher levels of polymorphisms when compared to allozymes, and can produce a much higher number of reproducible marker loci. Furthermore, the RAPD assay is much more cost effective and easier to perform than traditional microsatellite assays since no prior DNA sequence information is required. Under the right conditions, the RAPD banding patterns are very reproducible and easy to analyze. For this particular study, 5 individuals from each of 10 populations of *Z. strateumatica* were analyzed using RAPD markers.

Genetic parameter	Population										Mean
	SK	KT	YL	MOS	FL	KKL	JB	TSW	CU	UST	
p	1.49	0	1.49	4.48	2.99	2.99	2.99	0	8.96	2.99	2.838
A	1.015 (0.122)	1 (0)	1.015 (0.122)	1.045 (0.208)	1.030 (0.172)	1.030 (0.172)	1.030 (0.172)	1 (0)	1.090 (0.288)	1.030 (0.172)	1.029 (0.026)
A_e	1.007 (0.056)	1 (0)	1.007 (0.058)	1.028 (0.137)	1.014 (0.081)	1.028 (0.158)	1.021 (0.126)	1 (0)	1.062 (0.212)	1.028 (0.158)	1.020 (0.019)
S	0.008 (0.061)	0 (0)	0.008 (0.061)	0.025 (0.118)	0.015 (0.086)	0.020 (0.115)	0.018 (0.102)	0 (0)	0.053 (0.171)	0.020 (0.115)	0.017 (0.015)
H	0.005 (0.039)	0 (0)	0.005 (0.039)	0.017 (0.079)	0.010 (0.055)	0.014 (0.082)	0.012 (0.070)	0 (0)	0.036 (0.118)	0.014 (0.082)	0.011 (0.011)
$N_{\text{individuals}}$	5	5	5	5	5	5	5	5	5	5	5 (0)
N_{loci}	62	59	61	53	53	59	62	59	56	63	59 (4)

Table 1: Genetic variation within populations of *Z. strateumatica* based on RAPD data. p = percentage of polymorphic loci, A = number of alleles per locus, A_e = effective number of alleles per locus, S = Shannon index, H = Nei's gene diversity, $N_{\text{individuals}}$ = number of individuals sampled, and N_{loci} = number of loci. Standard deviations are given in parentheses

The results of this study helped to support the initial finding that the amount of genetic variation within specific populations of *Z. strateumatica* species was very low (Table 1). The average within population level of genetic diversity for this species was 2.838%. The within species averages from the other two sexually reproducing populations were 22% from *Z. gracilis* and 18% from *E. sinensis*. The actual within species rate of genetic diversity was very high within *Z. strateumatica*. However, this was largely attributed to the great extent of genetic divergence between individual populations. Therefore, single isolated populations of *Z. strateumatica* tend to have very little or no genetic diversity.

When compared to the sexually reproducing *Z. gracilis* species of orchid, the total amount of genetic diversity at the species level was very similar. However, as mentioned previously, this was largely due to the diversity between the individual populations of *Z. strateumatica*. Most of the RAPD markers studied within populations of *Z. strateumatica* were fixed. This lack of gene flow and genetic differentiation was consistent with what was expected of the apomictic species. Apomixis reduces sexual recombination within populations and gene flow through pollen among populations. The end result is populations with low genetic diversity and high genetic divergence. Furthermore, founder effects and genetic drift from these small populations of *Z. strateumatica* could lead to an increased fixation of genotypes which further enhances these population characteristics.

Genetic Diversity in Obligate Apomicts

In order to develop a population with the ability to adapt to new environmental stresses, a high level of genetic diversity is very important. This is particularly important in developing countries where factors like drought and novel pests are a continual worry. If a population becomes fixed for all alleles, and its only form of variation comes from

somatic cell mutations, it will not be able to rapidly adapt to a new stress. Plant species which are obligate apomicts are particularly vulnerable to this problem. They are essentially incapable of producing genetic variability. Therefore, alternative methods for the maintenance of genetic diversity must be employed in plant species reproducing this way.

One method for maintaining diversity would be to consider a population of obligate apomicts as a variety of unique hybrid genotypes (6). To accomplish this, multiple genotypic varieties of a specific crop line could be produced. Essentially, diverse populations could be developed that have many combinations of hybrids that are variable for certain characteristics and uniform for others. By commercially increasing the seed from many diverse obligate apomictic populations and mixing the seed prior to the purchase, a genetically diverse population would be insured.

Through this method, farmers would be able to reap the rewards of hybrid vigor while still maintaining a genetically diverse population capable of adapting to new stresses. Over time, the frequency of genotypes in this population may shift due to local factors. If this is something undesirable to the farmer, the original population could be purchased again and the genotypic frequencies would be returned to their original state.

Another method for maintaining genetic diversity in obligate apomicts, which would require several generations to truly take advantage of, would be to introduce apomixis genes into a very diverse sexually reproducing population. Sexual by apomictic crosses would generate sexual and apomictic hybrids which would have a selective advantage over the other members of the population. The best hybrids would increase in frequency and interpollinate to produce a superior population. Dominant genes that endow desired phenotypes would become fixed more quickly than traits which are controlled by a recessive allele. This method maintains genetic diversity and increases performance through continued cycles of recombination and fixation of desired genotypes.

Genetic Diversity in Facultative Apomicts

Ideally, if a single dominant gene could be isolated which controlled obligate apomixis, this would be the preferred method for development of superior cultivars. Furthermore, if genetic uniformity is the most important factor to consider, then obligate apomixis would be the more desirable trait to have. It also helps to maintain more of the original hybrid vigor originally seen from the hybrid.

However, if genetic homogeneity and morphological uniformity are not major concerns, then facultative apomixis is an option. These plants reproduce both sexually and asexually and the frequency of maternal progeny can range from low to high in plants with this type of reproductive strategy. For the most part, various levels of sexuality exist in most apomictic plant species.

The ability to introduce a gene controlling facultative apomixis into crop lines in developing countries could be very beneficial. After all, being able to capture some hybrid vigor is better than having none at all. There is an inversely proportional relationship between the level of facultative apomixis and the benefits received from it. The more facultative apomixis present, the smaller the rewards due to the increased capacity to generate variable progeny via sexual reproduction.

Facultative Apomixis in Guayule

Guayule is a small rubber producing perennial native to the Chihuahuan desert of north central Mexico and southwestern Texas. This plant has been regarded as a promising alternative source for natural rubber which the United States is totally dependent on foreign countries for its supply. The annual cost of importing all of this rubber is somewhere near \$1 billion (7).

In order for guayule to be a viable source of natural rubber, higher yielding cultivars need to be produced from the current stocks of germplasm. In order to produce these high yielding cultivars, a large amount of genetic diversity needs to be available for selection on traits such as rubber quality and quantity, dry weight, resin content, yield, and ploidy level.

Guayule reproduces primarily through apomixis. Therefore, it was always assumed that genetic uniformity would persist generation through generation of progeny. However, since guayule is a facultative apomict (not obligate), a large amount of genetic variation is seen in the progeny. For the most part, guayule populations are very heterogeneous and individual plants are very heterozygous in genetic makeup. Therefore, whenever sexual reproduction does occur, a large amount of genetic diversity is released. Furthermore, guayule has varying levels of ploidy within its populations. Polyploids normally reproduce through apomixis and diploids reproduce sexually. In the apomictic guayule, pollination is not necessary for embryo development, but is needed for normal endosperm development.

The facultative nature of apomixis in guayule results in four separate classes of progeny (Figure 3). Tetraploid parents ($2n=4x=72$) provide for a tremendous amount of complexity in reproduction. The majority of the progeny classes are tetraploid and identical to the maternal plant. These result from the non-reduction of the megaspore mother cell without fertilization. However, some progeny will increase in ploidy level to hexaploids ($2n=6x=108$) due to fertilization and an unreduced megaspore mother cell. Others decrease in ploidy to polyhaploid ($2n=2x=36$) plants from the reduction of the megaspore mother cell and embryo development without fertilization. The final class is the sexually reproducing class which comes from normal reduction and fertilization ($2n=4x=72$). Therefore, 2 progeny classes will remain tetraploid, one from apomixis and the other from sexual reproduction. The other two classes vary up or down in ploidy level from the original parent population.

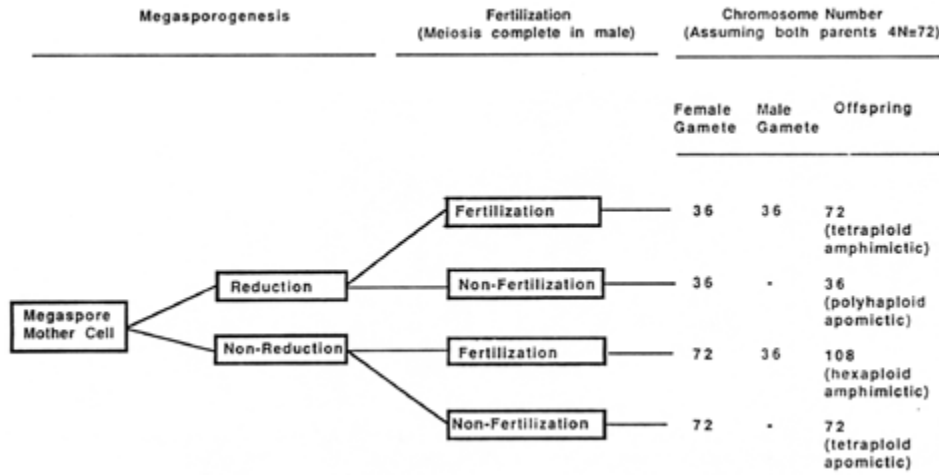


Figure 3: Reproductive systems in guayule which result in 4 progeny classes.

To quantify the amount of genetic variation present within the guayule population, 42 breeding lines were selected on the basis of superior yield, rubber concentration, and top regrowth. The selected plants consisted of four-year old progeny rows from single plant selections of facultatively apomictic plants. Ten plants selected at random from each of the 42 lines were studied. A total of seventeen characters were measured and described for each line. The variation discovered for these traits suggests that there is much variation still available to be exploited through selection. For example, the mean measurements from the 42 lines for rubber content ranged between 4.9 and 9.8% (Figure 4). Therefore, due to the facultative apomictic nature of the guayule reproductive process, it was concluded that there was enough genetic diversity present in the populations to produce the high yielding cultivars needed for this crop to be a viable source of natural rubber.

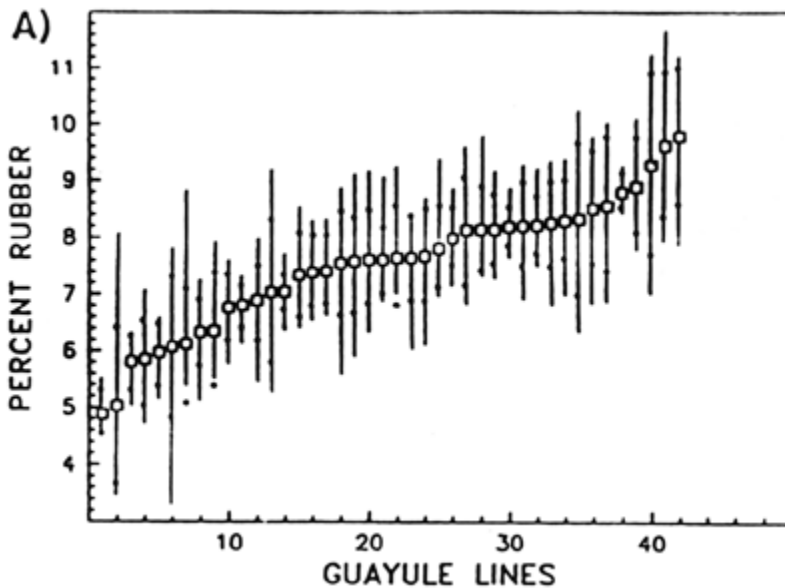


Figure 4: Variability for percent rubber content in 42 guayule lines.

Genetic Diversity and Release of Apomictic Varieties

The defining of molecular markers closely linked to apomixis could allow the coding region to be cloned and inserted into other crop species. In *Pennisetum*, 12 PCR amplifiable markers have been identified which segregate closely with an apospory specific region. There is a general lack of recombination in this region which could indicate that this apomictic coding region is part of a larger complex (6).

If genetic control of apomixis involves more than one gene or a complex of genes, or if there are many gene modifiers involved, then the transformation of current varieties into apomictic varieties would probably need to be controlled by professional breeders. From this, varieties with a complex genetic structure could be rendered apomictic and released. These complex varieties would help to maintain a high level of genetic diversity in the fields. However, if genetic control of apomixis is simple, introduction into landraces could be much easier to accomplish.

Two types of apomictic varieties can be distinguished: forage varieties which are already released as apomictic varieties, and apomictic varieties like maize and pearl millet which could be released in the near future (4). With the transferring of apomixis into pearl millet, maize, wheat, and rice, scientists are now forced to consider the consequences of such an event since apomixis would greatly reduce the rate of recombination. For these crops, the current landraces and their ancestors are all that remains for genetic diversity. Since apomixis fixes hybrid vigor, there are two possibilities for its use. The first is to produce apomictic F1 hybrids through breeding programs and release them to farmers. Secondly, farmers could be given the apomictic varieties to use in transferring the apomixis gene into their current crop lines. Eventually, all of those lines would be apomictic and the breeding of apomixis would become a local activity.

Conclusion

Perhaps the greatest irony of all is that most of the concern over genetic diversity is a result of the intense selection pressure applied by today's breeding programs. In these crops lines which are already depleted of a large amount of variation, apomixis would have the most problems. However, in developing countries where a large amount of genetic diversity is still prevalent, apomixis could have a very positive impact. This is especially true if apomixis is facultative. Since obligate apomixis is very rare and facultative apomixis is more common, this would most likely be the case. Even if a low level of sexual recombination was present in the population, new combinations could be generated at a significant rate from these highly diverse lines.

In general, as long as apomixis is not obligate, landraces can still evolve. The rate of evolution depends on the proportion of reproduction which occurs sexually. Therefore, this must be considered prior to the introduction of apomixis into a new cultivar. Furthermore, if the rate of sexual reproduction is under simple genetic control, it may be possible to manipulate. This could prove to be very valuable in maintaining genetic diversity and allowing for evolution of apomictic crops.

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