

GENETIC ANALYSIS OF APOMIXIS

Scientists and geneticists have studied the two broad categories of apomixis—gametophytic and sporophytic—because of their widespread occurrence and potential usefulness in plant breeding. The genetic analysis of apomixis provides researchers with unique obstacles because of ploidy levels, lack of sexual progeny, lethality, and accurate identification and classification of progeny.

Methods of accurately classifying progeny in genetic studies are being identified and currently several methods are available to researchers, none of which provide a complete picture. These include phenotypic analysis, cytoembryological study, microbiological methods, and a variety of markers. A combination of these methods may be used to accurately classify progeny.

Apomixis can be divided in two main types, gametophytic and sporophytic. The two types depend on the fate of the unreduced cells. If the unreduced cells give rise to a megagametophyte, then gametophytic apomixis occurs. If the unreduced cells give rise directly to an embryo, then sporophytic apomixis occurs (Spillane et al., 2001). The distinction between the two main types of apomixis is thus based on the site of origin and the subsequent developmental pattern of the cell which gives rise to the embryo. Figure 1 illustrates the comparison of sexual and apomictic pathways in angiosperm ovules. The important factor that distinguishes the sexual pathway from the apomictic pathway is the reductional division, or meiosis, of the unreduced somatic cells.

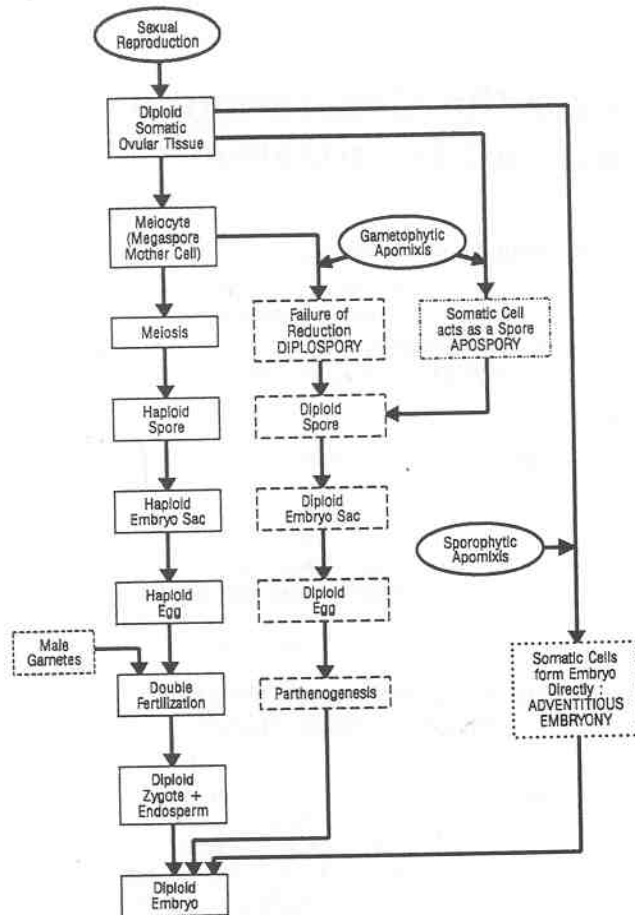


Figure 1: Comparison of sexual and apomictic pathways in angiosperm ovules with an emphasis on the differences between the two gametophytic pathways, apospory and diplospory, and the sporophytic pathway (Koltunow et al., 1995).

Sporophytic apomixis, also referred to as adventitious embryony, is a process in which the embryo arises directly from the nucellus or the integument of the ovule (Koltunow et al., 1995). The embryo development is initiated as a bud-like structure through mitotic division of the cell nucleus (Bashaw, 1980). The formation of the embryo sac is normal. Because it occurs normally, the embryo sac formation is not involved with apomixis. Sporophytic apomixis occurs commonly in *Citrus* species but rarely in higher plants. As such, there is much less information available regarding sporophytic versus gametophytic apomixis.

In the more common gametophytic apomixis, two mechanisms are generally recognized, diplospory and apospory. The two mechanisms are distinguished by the origin of the cells that give rise to the apomictic embryo. In diplospory, the embryo sac originates from the megaspore mother cell either directly by mitosis and/or after interrupted meiosis. In apospory, the embryo sac originates from nucellar cells. Apospory is the most common mechanism of apomixis in higher plants (Bashaw, 1980).

METHODS TO IDENTIFY APOMICTS

To identify plants used in the genetic analysis of apomictic species, researchers use phenotypic methods, cytoembryological characterization, genetic markers, biological tests, and whole plant testing (using a combination of methods) to determine the location of the gene(s) controlling apomixis in a viable crop species. These methods enable the classification of aberrant progeny derived from the cross of an asexual by sexual plant to precisely determine the mode of reproduction followed by each plant.

Phenotypic Identification

Phenotypic detection of apomixis involves the classification of a known heterozygous maternal parent and its progeny (grown from seed) with no observed segregation; or, in the case of facultative apomicts, the presence of several progeny with complete heterozygosity (that is, no known traits homozygous that were heterozygous in the maternal parent). Phenotypic classification may involve the least work of any technique for detection of apomixis, but is also the least reliable. Codominant or incompletely dominant alleles are necessary for proper classification of heterozygotes. Other factors, such as interference with meiotic crossing over, may make progeny appear to reproduce the maternal genotype when in fact they do not. The analysis of traits that cannot be classified visually (enzyme activity, starch production, etc.) may provide enough additional phenotypic information to greatly improve accuracy (Khokhlov, 1970).

In the absence of pseudogamy, careful emasculation and isolation of the maternal parent may also provide evidence of apomixis. In this case, if fertile seeds are produced in the absence of pollen, apomixis is likely present. In addition, the production of a high percentage of irregular, sterile pollen grains is a traditional, reliable apomixis indicator, but for autogamous plants only (Asker and Jerling 1992).

Cytoembryological Detection

Cytoembryological detection involves the observation of the developing spores and embryo to see whether reduction of genetic material occurs and to observe the nature of chromosome segregation in the formation of the megaspore, and to rule out the possibility of gametic fusion. This can be quite laborious, but does enable the detection and classification of the form of apomixis (Khokhlov, 1970).

Microbiological Methods

In the examination of apomixis, biological methods such as the auxin test, cytological tests, and ovule culture tests have proven useful in showing the characteristics of apomictic plant species. The auxin test can be used to demonstrate the estimation of the frequency of apomixis in Kentucky bluegrass (a facultative apomictic crop) (Sherwood, 2001). The test uses a synthetic auxin placed on the inflorescence before anthesis, which induces parthenogenic apomixis. Individual plants will develop grains that form mature embryos but no endosperm, whereas the opposite occurs for nonparthenogenic plants. The difference in the frequency between the two progeny plant types developed by the auxin test allows the estimation of apomictic capacity of the selected parents (Mazzucato et al., 1996).

Molecular and Conventional Markers

Conventional and molecular markers are used in mapping and gene discovery to identify loci controlling apomixis in apomictic plants (Spillane et al., 2001). Molecular markers, such as RFLPs, RAPDs, and AFLPs are screened onto segregating apomictic populations and their respective parents through different mapping strategies to detect any polymorphism that may be present. The problem with this technique, however, is that there are few traits specifically linked to apomixis that can be used as markers. Sherwood (2001) states that, “apomictic genes have no known effects on plant characteristics.” Until recently, this imposed a barrier in mapping apomictic species because a large number of markers were not available to provide linkage analysis information to map the apomictic gene region.

Conventional markers can be used for the analysis of progeny after a hybridization event between a cross of a facultative asexual species and sexual species. The use of markers is essential since three different sexual ‘off-types’ are frequently observed in the progeny of apomicts: (1) B_{III} hybrids, (2) B_{II} hybrids and (3) polyhaploids, (Spillane et al., 2001). Conventional, unlinked, monogenically inherited traits have been used as markers to distinguish maternal (asexual plants) from sexual (sexual off-type) plants (Sherwood, 2001). Conventional markers are useful if the asexual female parent is homozygous for a recessive trait and the pollen parent is homozygous dominant. Uniformity of the progeny for the asexual female parent marker would suggest maternal inheritance.

GENETIC BASIS FOR APOMIXIS

Theories of inheritance with apomicts have evolved over time. Early theories were dominated with ideas of polyploidy and hybridity. To reach these conclusions, researchers studied the nature and behavior of apomicts and noted most apomictic plants were polyploid and cytologically resemble species hybrids. Physiological factors and hormones were thought to be direct causes of apomixis. For example, one theory proposed wound hormones produced when plants were injured induced apomixis. Stebbins (1941) ruled out polyploidy as a cause of apomixis because sexual polyploids greatly outnumber the apomicts. Gustafsson in 1947 analyzed evidence for each theory of the time and concluded that no single phenomenon could be responsible (Bashaw & Hanna, 1990).

Due to the complexity of the apomictic system and the lack of inheritance data, it has been difficult to develop a single genetic explanation for apomixis. Several theories are currently being debated and can be divided into theories regarding apospory and diplospory.

Apospory

Studies on the Panicoidea indicated that one locus controlled apospory and a second independent locus affected sexuality. Burton & Forbes in 1960 demonstrated genetic control of apospory in *Paspalum notatum* using a novel hybridization technique. The analysis of these data indicated that a few recessive genes controlled apomixis (Bashaw & Hanna, 1990).

Taliaferro & Bashaw in 1966 investigated the inheritance of apomixis in buffelgrass. The resulting data indicated a mode of reproduction controlled by two genes with epistasis favoring dominant expression of the gene for sexuality (Bashaw & Hanna, 1990).

Hanna et al. (1973) used naturally occurring sexual tetraploids as female parents in crosses with natural tetraploid apomicts. Their results indicated that sexuality was dominant to apomixis and that the method of reproduction was controlled by two loci acting in an additive fashion (Bashaw & Hanna, 1990).

Sherwood et al. (1994) proposed an alternative analysis to the three previously mentioned studies. This was one genetic model involving two tetrasomically inherited loci. In this model the *A* locus is a dominant gene for apomixis and recessively lethal. The *B* locus has a dominant allele epistatic to *A* (Sherwood, 2001).

Nogler (1984a) suggests a monogenic inheritance for apospory where the induction of aposporous embryo sac from somatic nucellar cells is possible by an apospory factor *A*⁻. The wild allele *A*⁺ does not contribute to apospory but may function in the normal sexual life cycle. Nogler (1984a) noted that plants homozygous for *A*⁻ were unknown, suggesting lethality.

Savidan in 1991 and 1992 and Peacock in 1993 suggest a single master gene responsible for the induction of embryo sac formation (Sherwood, 2001). Induction would trigger events requiring direction by many genes and having a potential for modifying genes. Perhaps several genes relating to apomixis may be on a small chromosome segment or linkat. Jefferson in 1993 suggested apomixis involves phenotypic mutations at several loci acting together as a nonrecombining unit (Sherwood, 2001). This would give rise to the apomeiosis locus discussed by Spillane et al. (2001).

Diplospory

Characterization of diplospory is much more difficult than apospory on the basis of cytological evidence (Bashaw & Hanna, 1990). In mature ovules characterization is nearly impossible, as the embryo sac may appear completely normal. Cytological evidence of diplospory requires examination of early megasporogenesis, when normal meiosis would be expected. If mitotic metaphase is observed instead of meiosis in the megaspore mother cell, this indicates diplospory (Bashaw & Hanna, 1990).

Mitotic diplospory, also called the *Antennaria* type, is the more common type of diplospory. Less common is the restitutional diplospory, also known as *Taraxacum* type.

Mitotic diplospory

In *Eragrostis curvula* (weeping lovegrass, Poaceae), Voigt and Burson (1983) crossed naturally occurring sexual tetraploids with apomictic tetraploids and hexaploids. The F₁ progeny test segregations indicated that apomixis was a single gene dominant trait.

Sherman et al. (1991) studied apomixis in *Tripsacum dactyloides* (Eastern gamagrass, Poaceae). The authors crossed a sexual diploid female with a triploid apomict and examined the offspring. Based on cytological evidence, the authors believed an incompletely dominant pattern

of inheritance, or that minor additive genes affected the penetrance of apomixis. Leblanc et al. (1995) found the *T. dactyloides* tetraploid is simplex for the diplospory allele, based on the 1:1 segregation of maize-*Tripsacum* F₁s for mode of reproduction.

Working with *Parthenium argentatum* (guayule, Asteraceae), Gerstel and Mishance (1950) made reciprocal crosses between a sexual diploid and a facultatively diplosporous hyperdiploid. The results were less than conclusive. Gerstel and Mishance hypothesized a recessive but additive apomixis gene model. In addition, they concluded that polyploids with two apomixis genomes and one sexual genome were apomictic.

Nogler (1984b) performed a similar experiment but arrived at a different conclusion. Nogler theorized a single dominant gene controlling apomixis, but acting as a recessive lethal. The polyhaploid parents would then be *Aaa*, and the diploids *aa*.

Restititional diplospory

Theories regarding restititional diplospory involve studies of *Taraxacum* (dandelion, Asteraceae). According to Sherman (2001), eutriploids ($2n = 24$) and many hypotriploids ($2n=23$) have facultative diplospory. Interpretation of studies involving these types of plants has led to a more complicated hypothesis of the inheritance of apomixis. Mogie (1988) proposed that apomictic expression depended on one or more genes on one chromosome, and on dosage. Two copies of the mutant apomixis allele would be required to cause apomixis. In diplosporous apomicts, the allele would prevent meiosis. Mogie further suggested that the wild type (*a*) allele of the apomixis locus would have an essential function in the plant, perhaps coding for meiotic reduction and being involved in control of mitosis.

CONCLUSION

Molecular markers, microbiological tests, cytoembryological studies, phenotypic analysis, and combinations of these techniques have increased the ability to accurately identify and characterize the gene(s) controlling apomictic expression in apomictic plant species. Combined use of two or more of these approaches during genetic analysis of apomicts allows more precise results of apomictic gene(s) location and differentiation between progeny to be obtained.

Although the mechanisms of apomictic inheritance have been studied in relatively few species, there are several proposed models for its genetic control. Theories of inheritance range from a single apomixis locus or linkage group common to all apomicts to independent, random mutations at various reproductive loci. The truth about inheritance of apomixis appears to lie somewhere between these two extremes. Whatever the underlying mechanism, the genes affected by these loci are likely to play important roles in sexual development and understanding of apomixis (Spillane et al., 2001).

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