

Apomixis Sensu Stricto

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Apomixis may now be regarded as a consequence of sexual failure (i.e., loss-of-function) rather than as a recipe for clonal success (i.e., gain-of-function). There is increasing evidence that apomixis is a modification of the normal sexual developmental pathway. Most of the events that characterize sexual reproduction may be retained both structurally and functionally in apomictic reproduction, with the exceptions that the reduced egg cell is replaced by an unreduced egg cell, with absent or modified meiosis (i.e., apomeiosis), and the seed development does occur without egg cell fertilization (i.e., parthenogenesis). In addition, it is clear that residual sexual function is retained in pseudogamous apomixis, as seed development may occur without fertilization of either the egg cell or the central cell (i.e., autonomous apomixis), but fertilization may be required to form the endosperm in many apomictic plants.

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1. Introduction and Background Information

Apomixis *sensu stricto* (agamospermy) is asexual reproduction by seed ^[1]. For eukaryotes in general, apomixis is life cycle renewal through gamete-like cells that give rise to maternal embryos but without sexuality and sex, that is, without ploidy reduction (i.e., meiosis) and ploidy restitution (i.e., syngamy). Both meiosis and syngamy are canalizations of complex molecular processes that have remained conserved among single-celled and, later, multi-celled species of eukaryotes since eukaryogenesis ^{[2][3][4][5][6][7][8]}. Apomixis, like sex, is kingdom ubiquitous occurring in thousands of species across eukaryotes ^{[9][10][11]}.

Since apomictic reproduction involves the development of an embryo from an apomeiotic or somatic cell with a maternal genome, there are several ways to produce seeds of apomictic origin. This process can have a nucellar or integumental initiation, depending on the tissue of the ovule from which the maternal cell with embryonic competence differentiates.

The simplest pathway, known as adventitious embryony, avoids the production of a gametophyte and one or more vegetative embryos form within the nucellus or the integument. This phenomenon seems to have evolved more frequently in tropical than in temperate flora ^{[12][13]} and to be more represented in diploid species, while other forms of apomixis are more frequent in polyploids. Adventitious embryony is found in several non-agriculturally important species, with the exception of several *Citrus* species and mango (*Mangifera indica*) ^[14].

Another pathway, known as gametophytic apomixis, occurs when the maternal embryo originates from an apomeiotic egg cell differentiated into an unreduced embryo sac ^[15] arisen from a somatic nucellar cell that acquires the developmental program of a functional megaspore (apospory) or from a megaspore mother cell with suppressed or modified meiosis (diplospory). It is worth nothing that the gametic cell fate in apomictic plants can be activated either in somatic cells (apospory) or in unreduced megaspores (diplospory) as surrogate for meiotic products ^{[6][16]}.

Sexual reproduction is based on the alternation of a diploid (sporophytic) and haploid (gametophytic) generation, both of which are fringed by events that lead to a change in ploidy, i.e., meiosis and fertilization. In gametophytic apomixis, both edge events are short-circuited as the egg cells originate through mitosis (apospory) or restitutional meiosis (diplospory), preserve a maternal genomic composition, and the embryos develop autonomously without any contribution of a spermatoc nucleus (parthenogenesis). This combination was referred to as recurrent apomixis ^[15] as the original genotype may be indefinitely reiterated over generations.

Scaling up and down the ploidy level by means of genome accumulation or limitation can eventually take place by unreduced egg cell fertilization or reduced egg cell parthenogenesis respectively. These reproductive strategies have been referred to as non-recurrent apomixis ^[15]. Although not offering a stable means for genotype propagation, apomictic variants have likely been an important player in the evolution of polyploid species.

2. The Genetic Control of Apomixis: A General Overview

One of the major challenges of population biology is to understand which are the genetic determinants that control the maintenance of sexual reproduction under natural selection. In this view, the fundamental components of amphimixis, such as genetic recombination and gamete fusion, allow the single individuals of a population to experience new allelic combinations and interactions leading to diversification and adaptation. Nevertheless, alternative routes of reproduction that circumvent sex, such as apomixis, gained significant evolutionary success ^[17]. In the offspring of apomictic plants, genetic diversity is avoided or minimized, as the embryos retain the maternal genotype and their development is independent from both meiotic reduction and egg cell fertilization in ovules. Fertilization of the central cell is often required for endosperm formation (pseudogamy).

During the last two decades, many scientists have worked on the isolation of the genetic determinants of the apomictic pathway with the perspective to induce apomictic reproduction in crop plants by genetic engineering (for review, see ^[16] ^[18]). More recently, although artificially induced apomictic rice has been obtained ^[19] using a synthetic approach (e.g., by engineering key regulator genes of sexual development), additional research is required to determine stability of induced apomictic reproduction in field conditions. Consequently, even now using the modern tools of genomics, understanding the molecular pathway leading to apomixis in natural apomicts is necessary, but more complicated than expected.

Nowadays, new insights have contributed to shed light into the structural and functional feature of apomixis; these include the structural parallelism between the apomixis controlling region (ACR) in several natural apomicts and the Y-chromosome of dioecious plants ^[18], the silencing mechanism of a specific apomixis-linked genetic factor ^[20] and the functional validation of a genetic determinant of parthenogenesis in *Pennisetum squamulatum* ^[21].

Recent advances, based on sequencing, in silico mapping and in vitro expression analysis of selected apomixis-linked genes allowed the identification of a chromosome area common to *Sorghum bicolor*, *Setaria italica*, *Brachypodium distachyum*, rice and maize syntenic to the apomixis locus of *Paspalum simplex* ^[22]. This synteny group revealed different extents of gene collinearity with the apomixis locus, including genes with well-defined annotations for biological processes and molecular functions. Most importantly, apomixis-linked genes were expressed as both sense and antisense mRNAs and both transcripts proved to be more abundant in sexual compared to apomictic ovules, indicating a putative silencing effect of the apomixis-linked alleles on their sexual-specific counterparts in these cells ^[22]. This finding could act in favour of apomixis surfacing or evolved by silencing sex genes.

More specifically, it would appear that there are at least two distinct elements in the control of gametophytic apomixis: the production of unreduced embryo sacs, originating through apospory or diplospory, and the subsequent development of the embryos through parthenogenesis. However, other elements may need to be incorporated into a model explaining/mimicking the genetic control of apomixis, including pseudogamy and, in some apomicts, the autonomous development of endosperm.

Apomeiosis is rather a rare phenomenon when uncoupled with parthenogenesis while it is frequently observed as formation of unreduced gametes to overcome sterility of inter-specific hybrids leading to the origin and evolution of polyploid forms ^[23]^[24]^[25]. Conversely, when apomeiosis is coupled with parthenogenesis it attains regular elevated expression in natural apomicts ^[26]. Furthermore available data suggest that in some cases apomeiosis, by either apospory or diplospory, may be functionally and genetically independent from parthenogenesis and autonomous endosperm formation ^[16]^[18]. Finally, in most apomicts, both apospory and diplospory proved to be simply inherited in populations segregating for apomixis and a complex genetic model based on delicate interactions between initiators and repressors of both apomeiosis and parthenogenesis has been proposed for several species ^[27]^[28].

Analysis of genetic and molecular studies of apomicts is provided by several reviews, including those of Ozias-Akins and Conner ^[29], Hojsgaard ^[30], Whitton et al. ^[12], Barcaccia and Pupilli ^[18] Hand and Koltunow ^[1] and Schmidt ^[31]. Both naturally occurring and induced mutants holding individual components of apomixis have been identified (e.g., ^[32]^[33]), meaning that many taxa can potentially express apomixis-related traits, and that each component is under independent control and regulation. In several natural apomicts, the existence of genotypes which express only one component of apomixis or suppress the other (reviewed by ^[16]), further supports the hypothesis that distinct genetic factors control apospory, diplospory and parthenogenesis. It has long been recognized that apomixis is under control of single-dose dominant alleles (if hypothetically, apomixis were to be controlled by recessive alleles, then multiple-copies would be necessary in polyploids). Indeed, apomixis is inherited as a dominant trait in several apomictic species (see ^[34], for a review). However, even in species with simple inheritance patterns it is doubtful whether a single gene controls apomixis. As a matter of fact, the occurrence of genotypes that form embryos either from fertilized apomeiotic egg cells or by

parthenogenic development of meiotically reduced egg cells have been documented by cyto-histological and flow-cytometric analyses, suggesting that apomeiosis and parthenogenesis may be uncoupled [16]. Recombinants for apomixis components that lack either apomeiosis or parthenogenesis have been reported in *Taraxacum officinale* [35], *Erigeron annuus* [36], *Poa pratensis* [37][38], *Hypericum perforatum* [39], *Ranunculus* [40] and *Cenchrus* species [41]. Finally, autonomous endosperm formation segregates independently from the other components of apomixis in *Hieracium*, [42][43]. On the whole, these findings suggest that apomixis may be controlled by a complex of closely linked genes.

A theoretical scenario for the origin and evolution of a two-gene apomixis system was proposed by Van Dijk and Vijverberg [44], including two dominant mutations that occur in a population of outcrossing hermaphrodites. A plant is changed by a first mutation from meiotic into apomeiotic, and by a second mutation from zygotic to parthenogenic embryo development. The chance that these two mutations would occur soon after each other within a nascent apomictic population seems unlikely [12]. There is, however, another possibility. Since most apomicts are of hybrid polyploid origin, perhaps the two mutations needed for the functioning of apomixis may be separately present in polyploid parent stocks and brought together by hybridization, leading to functional apomicts [45]. For the rise of apomixis in natural populations, an additional hypothesis calls the stepwise evolution model into question, as proposed by Hojsgaard and Hörandl [46]. It could be shown that sexual diploids have latent alleles for parthenogenesis with little or no penetrance, which become important benefiting from high expressivity if apomictic behaviour was introduced by specific mutations, giving rise to the development of apomeiotic egg cells. Moreover, for successful hybridization, a strict parthenogenic plant cannot function as a seed parent, since these mutations can only be combined in crosses between an apomeiotic seed parent and a parthenogenic male parent. This automatically results in polyploid apomictic hybrids, suggesting a direct relationship between gametophytic apomixis and polyploidy. New apomictic plants can function as a pollen donor in crosses with sexual plants, thereby generating new, secondary apomictic clones. This way would help explaining high clonal and genetic diversities, commonly found in populations of apomicts [47]. However, the main problem remains with this evolutionary scenario that the mutations for apomeiosis and parthenogenesis are individually deleterious and so they are expected to be selected against [48].

As reported by Briggs and Walters [45], devising a universal model of apomixis may be unrealistic: clearly this asexual mode of reproduction occurs in different forms and variants, and in many unrelated families of monocots and eudicots, suggesting that apomixis as a whole has evolved multiple times in angiosperm plant evolution following different developmental pathways, which perhaps are controlled by distinct genetic factors.

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