Chapter 14

Apomixis: Mechanism and Its Agricultural Potentials

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Asexual reproduction is referred to as apomixis, which occurs when plants acquire the ability to circumvent two of the most crucial processes involved in sexual reproduction: meiosis and fertilization. Despite the lack of male fertilization, the seed will germinate and grow into a maternal clone. The apomixis process has been observed in a large number of flowering plant species despite the fact that no major seed crops have been proven to be capable of apomixis. Scientists may be able to expedite agricultural breeding procedures if they were able to make maternal clones and, as a result, quickly fix desired genotypes in crop varieties. It is possible that apomixis will be used as a breeding technology for the next generation, which is one of the reasons why interest in the mechanisms controlling apomixis is increasing. The purpose of this chapter is to discuss recent developments in genetic and molecular research pertaining to the management of apomixis. There are currently two lines of research being pursued. It is the first objective of the study to identify and describe the genes responsible for apomixis in apomictic species that have been cultivated as model organisms. The second method involves modifying or otherwise altering the sexual seed generation process in non-apomictic animals in order to achieve results similar to those of apomixis. A review of the major apomictic mechanisms, as well as an update of our knowledge of the loci that control these mechanisms, is presented in this chapter. Additionally, it is about the study of candidate genes that may be useful in changing crops from sexual to asexual reproduction.

Keywords: *Apomixes, breeding technology, reproduction, meiosis, fertilization*

INTRODUCTION

Apomixis (asexual reproduction through seeds) [1] has long been seen as an unprecedented natural tool to maximize plant breeding, with potential wide impact on global farming systems [2]. In close developmental connection with sexuality, it functions as either a digressed or a parallel pathway, ending in the generation of clonal embryos of maternal origin within viable seeds [3]. Besides the importance of understanding this puzzling reproductive mode to advance the basic knowledge on reproduction, the combined use of sexuality and apomixis could accelerate the generation of novel improved plant varieties while dramatically diminishing the cost of the process, turning the notion of customized, locally-adapted hybrid crops adapted to every farm plot into reality [2]. The potential benefits of harnessing apomixis vary from full exploitation and permanent fixing of heterosis to seed distribution for crops actually propagated vegetatively like potatoes or strawberries [4]. However, the effective use of this trait in plant breeding requires full knowledge of the molecular switch allowing the transition from sexuality to clonal seed reproduction.

In the last 30 years, coordinated international efforts have led to the elucidation of major molecular actors controlling apomictic development [5]. The integration of these genes into interlaced functional pathways is currently under investigation, in the prospect of generating optimized biotechnological tools. With this aim, modulating the expression of some critical genes has allowed the rewiring of apomixis components in sexual plants [5] and even induced the production of clonal seeds in rice [6,7,8]. However, the stability of synthetic apomictic rice remains to be tested by natural selection and validated in field conditions. In any case, the molecular triggers of apomixis in natural agamic complexes (i.e., species composed of diploid sexual cytotypes and polyploid apomictic counterparts) remain unknown. Moreover, the harnessing of the trait into plant breeding acquired an entire new dimension under the proposal that apomixis and sexuality might be ancient polyphenic phenotypes, with both pathways represented in all plant species, although many lineages have lost the capacity to shift from one to the other [9]. This hypothesis implies that sexual species (like major crops) could become apomictic by restoring the lost natural switch between both phenotypes, provided that the asexual route remained operational. In order to identify the natural triggers of apomixis, extensive reproductive characterization should be conducted in a high number of taxa, to extend our knowledge on sexual and asexual reproduction coevolution. Up to date, although apomixis was reported in at least 78 of the 460 angiosperm families [10,11,12], only a few species have been

characterized from a molecular perspective. Moreover, less than 10% of the >350,000 flowering plant species have been examined using cytoembryological techniques, which suggests that new members previously assumed to be sexual might be added to the apomicts list soon [9]. Thus, there is a need to complete the information available on the cytoembryological, developmental, and molecular aspects of apomixis through the scrutiny of previously uncharted species, a task that should be ideally carried out by scientists from different countries, who have access and are familiarized with unique local materials collected from plant populations growing in their natural habitats.

Paspalum L. is one of the few genera in which sexual and asexual reproductive behaviors have been characterized side by side for more than 50 years. During this period, many approaches proved to be unsuccessful, while others offered significant advances. In this review, we aim at presenting the rationale supporting the work carried out in this particular genus, and, based on our previous experience, proposing a series of advisable steps that could help to explore the molecular control of the trait in other species, through genetic, genomic, and/or breeding approaches. We expect to favor the development of other research projects, in order to boost the investigation of this biologically amazing and complex field.

2. Developmental features of apomixis and sexual reproduction

During normal sexual reproduction, the megaspore mother cell (MMC) divides into four reduced megaspores through meiosis. Three of these megaspores undergo apoptosis and the remaining functional megaspore develops into a seven-celled, eight-nucleate embryo sac, consisting of one egg cell, one central cell, two synergid cells, and three antipodal cells. When the pollen tube penetrates into the embryo sac, double fertilization occurs [13]. One sperm cell fuses with the egg cell to form a zygote, while the other sperm cell fuses with the central cell and then develops into endosperm, which provides nutrients for embryo development. In the process of sexual reproduction, meiosis ensures the formation of a reduced embryo sac. Double fertilization not only produces the zygote and triploid nucleus, but also activates the initial development of the zygote by complicated signals from both egg cell and sperm cells [14, 15].

Compared with sexual reproduction, apomixis alters several steps during the initiation and formation of the female germline and produces an asexual embryo with a genotype identical to that of the mother plant . Based on the origin of the embryo, apomixis can be divided into two types, gametophytic

apomixis and sporophytic apomixis (adventitious embryo) [14]. Gametophytic apomixis refers to the asexual embryos derived from the unreduced embryo sac, which can be further divided into diplospory and apospory according to the origin of the cell that initiates unreduced embryo sac formation [15,16]. In diplospory, the MMC undergoes a modified meiosis and divides into two non-reduced megaspores. One of the unreduced megaspores develops into a diploid embryo sac, in which the diploid egg cell can directly develop into a parthenogenetic embryo without double fertilization [17]. In apospory, a nucellar (somatic) cell near the MMC acquires a gametophytic fate and directly gives rise to the gametophytic lineage without meiosis. The apomictic germline lineage can repress the development of the sexual gametophyte and form an unreduced embryo sac, in which a parthenogenetic embryo is directly developed from the diploid egg cell without fertilization. In some cases, many aposporous initial cells occur in a single ovule and develop into more than one aposporous embryo sac [18,19]. Gametophytic apomixis completely replaces amphimixis, and is regarded as obligate apomixis [20], while in most apomictic plants both sexual and asexual reproduction processes occur simultaneously in the same ovule, which is termed facultative apomixis [21]. In both diplosporous and aposporous ovules, the endosperm can develop spontaneously without fertilization or through pseudofertilization, providing nutrients for the development of the embryo [22].

For sporophytic apomixis, adventitious embryos are developed from nucellar or integument cells and coexist with the zygotic embryo, leading to the development of a polyembryonic ovule [23]. Generally, the adventitious embryo initial cells appear to be morphologically distinguishable after the formation of the sexual embryo sac. Then they enter the sexual embryo sac and compete with the sexual embryo for nutrients. The survival of the adventitious embryo depends on the fertilization of the sexual embryo sac, which can offer important nutrient and growth signals from the fertilized endosperm [24].

3. Apomixis Mechanisms

In contrast to sexual seed formation, apomixis can occur by various mechanisms that share three common developmental components: (i) a bypass of meiosis during embryo sac formation (apomeiosis), (ii) development of an embryo independent of fertilization (a process known as parthenogenesis), and (iii) formation of viable endosperm either via fertilization-independent means or following fertilization with a sperm cell (Koltunow and Grossniklaus 2003). Derivation of the egg from a diploid maternal cell without meiotic

reduction, and its subsequent fertilization-independent development into an embryo, means that the progeny derived from apomictic development are clonal and therefore genetically identical to the maternal parent.

Apomixis mechanisms are historically subdivided into two categories and classified as either gametophytic or sporophytic, based on whether the embryo develops via a gametophyte (embryo sac) or directly from diploid somatic (sporophytic) cells within the ovule [25] . During sporophytic apomixis, development of an embryo sac following the typical angiosperm sexual pathway still occurs. However, during mitosis of the functional megaspore, diploid somatic ovule cells surrounding the embryo sac differentiate and have an embryogenic cell fate. These embryo initial cells begin mitosis forming multiple globular-shaped embryos that can develop to maturity only if the sexually derived embryo sac is fertilized, as the sexual and asexual embryos share the nutritive endosperm. Sporophytic apomixis can therefore lead to a seed containing multiple embryos and is common in citrus. The sexually derived embryo may or may not mature or germinate [26]. Sporophytic apomixis has not been extensively studied at the molecular level; however, it appears genetically complex [27]. The remainder of this review focuses on gametophytic apomixis. Gametophytic apomixis relates to mechanisms where an embryo sac is mitotically formed from a diploid cell in the ovule, bypassing meiosis. Another term for such mitotic embryo sac development is apomeiosis. Embryo development in gametophytic apomixis is fertilization independent whereas endosperm formation may or may not require fertilization . Apomeiotic embryo sac development is further subdivided into two types (diplospory and apospory) based upon the origin of the diploid precursor cell that ultimately gives rise to the mitotically derived embryo sac. In diplospory, the precursor is the megaspore mother cell (or a cell with an altered program that differentiates in the megaspore mother cell position) . This cell may enter meiosis and abort the process or it may immediately begin mitosis. Diplospory has been observed in species including Taraxacum officinale (dandelion), Boechera spp., Erigeron annuus, and Tripsacum dactyloides. By contrast, apospory involves development of the embryo sac via mitosis not from the megaspore mother cell, but from a diploid somatic cell positioned adjacent to the megaspore mother cell . This cell, termed the aposporous initial cell, undergoes mitosis and the nuclei cellularize. The mitotic events of diplospory and apospory may or may not make a seven-nucleate Polygonum-type embryo sac; however, an egg, a central cell, and synergids are typically formed. Depending on the species, both sexually

derived and aposporous embryo sacs can coexist within the one ovule, as occurs in Brachiaria species [28]. Alternatively, development of the aposporous embryo sac may lead to the demise of the sexually derived embryo sac or pathway as occurs in aposporous Hieracium and Pennisetum species [29]. Embryo development from the diploid egg formed in aposporous and diplosporous embryo sacs occurs without fertilization and the term parthenogenesis is used to describe this process. Endosperm development can occur without fertilization of the central cell, although this is rare, occurring predominantly in members of the daisy family (Asteraceae). Apomicts that require fertilization to produce endosperm have disturbed maternal and paternal genome contributions (m:p) in the endosperm. For example, in such apomicts, fertilization of a tetraploid central cell may lead to a 4m:1p endosperm genome ratio, in contrast to the typical 2m:1p ratio of fully sexually reproducing species. Those apomicts that require fertilization to develop endosperm have therefore developed multiple strategies to ensure seed viability and these have been discussed in previous reviews[30].

4. Apomixis-controlling loci and related genes

From an evolutionary perspective, apomixis may have evolved from the same molecular framework as that which supports sexual reproduction. When sexual reproduction is aborted as a result of the mutation of corresponding genes, apomixis occurs to overcome infertility. In Arabidopsis, a set of mutants have been reported to display phenotypes resembling apomixis (Table 1), such as ago9 [30] and swi1 [31], which participate in chromatin remodeling; spo11-1/2 [32, 33], mtopVIB [34], dfo [35], prd1 [36], and rad50 [37, 38], which are involved in double-strand break formation; dmc1 [39], msh4 [40], and asy1 [41], which are essential for chromosome synapsis; rec8 [42], scc3 [43], and ahp2 [44], which are involved in the first meiotic division; osd1 [45] and tam [46, 47], which are related to the meiosis I–meiosis II transition; tdm1 [48], which controls meiotic termination after meiosis II; msi1 [49], which is able to initiate parthenogenetic development; cenh3 [50], which can induce haploid formation; and fie [51] and fis [52], which can induce endosperm development without fertilization. Most apomictic plants are facultative, which offers the possibility of genetic analyses of apomixis. In all species studied so far apomixis has been proved to be heritable. In citrus and mango, inheritance of sporophytic apomixis as single dominant locus has been proposed [16, 53], while in some diplosporous apomicts genetic loci controlling the key steps of apomixis (apomeiosis, parthenogenesis, and automatic endosperm development) are independent of each other. For example, two separate loci

that control diplospory and parthenogenesis have been identified in Erigeron and Taraxacum species [54, 55]. Apospory and parthenogenesis are determined by two different loci in Hypericum [56], Poa [57], and Cenchrus [58] species. In Hieracium, three independent loci, LOA, LOP, and AutE, have been discovered to control apospory, parthenogenesis, and autonomous endosperm development, respectively [59, 60].

Despite the discovery of multiple apomixis-linked loci in various species, it is still difficult to identify the specific genes controlling apomixis, as the apomixis-linked loci are usually recombination-inhibited and located in repetitive regions [61–63]. So far, a few genes have been identified that are involved in different components of apomixis (Table 1). For apomeiosis, two different candidate genes, APOLLO (apomixis-linked locus) and UPGRADE2 (unreduced pollen grain development), have been identified in Boechera. The expression of APOLLO and UPGRADE2 is strongly correlated with the formation of apomeiotic eggs and pollen, respectively [64–66]. In Tripsacum, AGO104, which is involved in DNA methylation, is proposed to be required for proper chromatin condensation during meiosis [67]. In Oryza sativa, the PAIR1 gene was identified to play an essential role in chromosome synapsis in early meiotic prophase [68]. For apospory, a MAP3K-coding QUI-GONJINN (QGJ) gene in Paspalum notatum is suggested to be essential for aposporous embryo sac formation [69]. In Brachiaria brizantha, the specific expression pattern of GIBBERELLIN-INSENSITIVE DWARF1 (GID1) suggests its function in aposporous initial cell differentiation to form the aposporic embryo sac [70]. In apomictic Hieracium, transient downregulation of a floral organ-identity gene (DEFICIENS) in the chalazal region is associated with aposporous initial cell formation [71]. Similarly, in P. notatum, PnTgs1-like was proposed to play an important role in nucellar cell fate, as its reduced expression is associated with the initiation of the aposporous pathway [72]. In Poa pratensis, PpSERK is proposed to be responsible for the formation of the aposporous initial cell and the development of the asexual embryo sac [73]. For autonomous endosperm formation, ORC3 and FIE were proved to be vital candidate genes. The accurate expression of ORC3 in germ cell lineages determines the development of the endosperm in apomictic Paspalum simplex [74]. In Malus hupehensis, FIE is involved in the regulation of asexual seed formation [75]. Ectopic expression of MhFIE in tomato produces parthenocarpic fruit [76]. For parthenogenesis, ASGR-BBML has been proved to be the most promising candidate. ASGR-BBML is expressed in unfertilized egg cells of apomictic Pennisetum squamulatum and transformation of sexual pearl millet with the ASGR-BBML gene can trigger

parthenogenesis [12, 77]. Recently, a PARTHENOGENESIS (PAR) gene was isolated from apomictic common dandelion, which can induce embryo-like structures without fertilization in lettuce [78]. In addition, mutation of a pollen-specific phospholipase, MTL1, can induce paternal genome elimination and haploid formation in maize and rice [79]. For adventitious embryogenesis, several candidate genes have also been reported. In citrus, the CitRWP gene was identified by genetic analysis of segregating populations and proved to be associated with nucellar embryo formation [80, 81]. In another typical sporophytic apomictic plant, Zanthoxylum bungeanum, the expression of AGL11 shows correlation with nucellar embryo development and its ectopic expression can lead to abnormal flower development and simulate apomixis phenotypes in Arabidopsis [82].

5. Are Stress Signal and Nutritional State Triggering the Determination for Sexual Reproduction or Apomixis?

Sexual reproduction and apomixis are classically viewed as two alternative types of reproduction with their own evolutionary histories. Recent ideas and insights challenge this perception and consider that both reproductive strategies might be polyphenic [18]. In this view, both modes of reproduction can be temporarily activated based on environmental conditions, stress, and nutritional state [18]. Evidence for the potential of a stress induced switch from apomixis to sexuality is given for a number of apomictic systems including Boechera, Paspalum, Ranunculus, and Eragrostis [18]. This might resemble an ancient mechanism. It has been hypothesized that the evolution of sexuality is a result of reactive oxygen species (ROS) that were generated starting with the development of primitive mitochondria at the basis of the evolution of eukaryotes [158,159]. From this perspective, the necessity for sex would be the consequence of ROS induced DNA damages as it allows purging of deleterious mutations from the genome [158]. Oxygen based DNA damage might have been also a requirement for meiosis to evolve and thereby lay the foundation for sexual reproduction [160]. It has been hypothesized that interactions of oxidized DNA and the core meiotic gene SPO11 has enabled double strand breaks and meiotic recombination to occur [160]. Curiously, however, in maize anthers, low levels of ROS promotes acquisition of meiotic fate [161]. From a recent study in A. thaliana, repression of the homeobox gene WUSCHEL (WUS) is important for the acquisition of meiotic fate by the MMC [162]. Relieving the repression of WUS activity in the MMC causes mitotic divisions before the cells eventually enter meiosis [162]. Interestingly, in the shoot apical meristem WUS activity is activated by ROS [163]. It might be

speculated that also in the maize anthers WUS regulation might be involved in determination of meiotic fate in response to ROS levels, if similar to regulatory processes are active in reproductive tissues. Furthermore, ROS has an impact on epigenetic regulatory systems and global DNA-methylation, suggesting the integration of stress signals and epigenetic regulation to control reproduction [18].

Consistent with a role of ROS to trigger meiosis, recent evidence suggests that redox regulation differs in sexual MMCs and the AICs (Figure 3A). In Boechera gunnisoniana, enrichment of polyamine and spermidine synthesis is a characteristic feature of the AIC [80]. This is in line with the identification of spermine/spermidine synthase from the ASGR in Pennisetum squamulatum [49]. The importance of the polyamine spermidine to protect the DNA from oxidative damage by scavenging of free radicals arising mostly from ROS has long been described [164]. Potentially, the importance of detoxification of ROS in the AIC is a consequence from the absence of meiosis. Such mechanisms to prevent deleterious mutations to arise by oxidative stress appear to be relevant particularly in the founder cells of the apomictic germline lineages. Interestingly, in Boechera nucelli tissues an UDP-glycosyltransferase superfamily protein is significantly higher expressed in all sexual as compared to all apomictic accessions analyzed [103]. While the functional role of this gene has not been investigated, it might be involved in synthesis of callose, as shown for certain members of this gene family [165]. Callose deposition is promoted by ROS [166], further supporting the idea that redox stress plays different roles for meiosis and apomeiosis, particularly as callose is typically not enclosing the AIC in contrast to the MMC [167,168] (Figure 3A). Thereby, callose deposition around the MMC might either shield the surrounding cells from ROS and its effects, or might be effective in protection of the meiocyte from disturbances. Future molecular studies are required to shed light onto this question. Nevertheless, the connection of ROS and callose further supports the idea of the importance of redox state for mode of reproduction.

Apart from ROS other types of stress like nutritional starvation and abiotic stress conditions including cold and heat have a great and versatile impact on meiosis and reproduction. Thereby, not only a shift from apomixis to sexual reproduction occurs, but also alterations of meiosis concerning recombination frequencies or the formation of unreduced or aneuploid gametophytes [169]. A central integrator of nutrient, energy, and stress related signals to regulate cell growth and development in eukaryotes is the target of

rapamycin (TOR) kinase. In the yeast Schizosaccharomyces pombe nutritional starvation triggers the onset of meiosis and sexual reproduction dependent on the activity of TOR pathways [170]. Recent evidence suggests an evolutionary conservation of these pathways, as application of glucose at a certain developmental time point leads to features of apomeiosis in sexual A. thaliana [171]. The underlying molecular mechanism remains to be investigated in detail. An interesting question will be if WUS activity in the MMC is elevated by application of glucose, a mechanism described for the shoot apical meristem [172]. If so, this might be the molecular mechanism of obtaining mitotic divisions of the MMC similar to previous reports on de-repression of WUS activity in the MMC [162].

Interestingly the TOR pathway also coordinates ribosome activity [173], implying a connection between stress, nutritional state, reproduction and cell cycle. Further ribosome biogenesis factors like RNA helicases are not only involved in the regulation of gene activity and developmental decisions, but also in mediating stress response and growth regulation [126]. Functional implications in stress response have in particular been described for a number of RNA helicases, including AtRH36 involved in regulation of gametogenesis and ENHANCED SILENCING PHENOTYPE3 that has previously been described to be active in the AIC in Boechera gunnisoniana unlike in the A. thaliana MMC [80,126]. Also heat shock proteins are stress responsive proteins tightly associated to ribosome function, as they typically assist folding of newly derived polypeptide sequences to proteins as chaperones. Evidence for roles of heat shock proteins in apomixis regulation comes from different types of apomixis, including apospory in Hieracium prealtum [34], apomixis in Paspalum notatum [92], somatic embryogenesis in Citrus [76], and apogamy in the fern Drypteris affinis [174]. In addition, it is interesting to note that AP2/ERF transcription factors are important players in the integration of hormonal pathways and stress responses to control developmental decisions [154].

The regulation of reproductive development related to environmental factors and nutrition represents a conserved mechanism in eukaryotes. It can easily be envisioned that particularly in largely facultative apomictic systems such factors allow us to modify the frequencies of apomixis. Nevertheless, the heritability of apomixis and the identification of the genetically linked loci suggests the requirement of certain genetic elements for apomixis.

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