Mini Review

Apomixis: genetic basis and controlling genes

Yuantao Xu, Huihui Jia, Chunming Tan, Xiaomeng Wu, Xiuxin Deng and Qiang Xu*

Key Laboratory of Horticultural Plant Biology (Ministry of Education), Huazhong Agricultural University, Wuhan, Hubei 430070, China
*Corresponding author. E-mail: xuqiang@mail.hzau.edu.cn

Abstract

Apomixis is the phenomenon of clonal reproduction by seed. As apomixis can produce clonal progeny with exactly the same genotype as the maternal plant, it has an important application in genotype fixation and accelerating agricultural breeding strategies. The introduction of apomixis to major crops would bring many benefits to agriculture, including permanent fixation of superior genotypes and simplifying the procedures of hybrid seed production, as well as purification and rejuvenation of crops propagated vegetatively. Although apomixis naturally occurs in more than 400 plant species, it is rare among the major crops. Currently, with better understanding of apomixis, some achievements have been made in synthetic apomixis. However, due to prevailing limitations, there is still a long way to go to achieve large-scale application of apomixis to crop breeding. Here, we compare the developmental features of apomixis and sexual plant reproduction and review the recent identification of apomixis genes, transposons, epigenetic regulation, and genetic events leading to apomixis. We also summarize the possible strategies and potential genes for engineering apomixis into crop plants.

Introduction

Generally, angiosperms go through sporophytic and gametophytic generations alternately, and produce future generations by sexual reproduction. However, some plants can also reproduce asexually by apomixis. Apomixis is an asexual reproduction process that produces seeds in the absence of meiosis and fertilization [1, 2]. As apomictic plants can produce clonal offspring that fully retain the genotype of their mother plant through seeds, apomixis can provide many agronomic advantages for crop production: the stable fixation of heterosis through seed; the rapid generation of new superior germplasms; the simplification of hybrid seed production procedures; and the purification and rejuvenation of some vegetatively propagated varieties, such as perennial woody fruit trees [3]. Applying apomixis to the seed production of crops will drive a new green revolution in agricultural science [4].

Apomixis was initially discovered in *Alchornea ilicifolia* [5], and subsequently had been described in more than 400 flower plant species [6]. Many important genera in Asteraceae and Poaceae are reported as typical apomictic plants, such as *Hieracium*, *Taraxacum*, and *Pennisetum*. Some species in these genera are widely studied to dissect the genetic control of apomixis [7–12]. Apomixis also occurs widely in horticultural crops, including citrus [13], crabapple [14], walnut [15], mango [16], pepper [17], and Chinese chive [18]. However, apomixis is relatively infrequent in major crop species [6]. Understanding the mechanism and control of apomixis in existing apomictic plants is the prerequisite for applying apomixis in agriculture.

In this review, the developmental features of apomixis and sexual plant reproduction are described. We summarize the recent understanding of the factors influencing apomixis, including genetic control, transposons, epigenetic regulation, polyploidization, and hybridization. We also propose possible strategies and potential genes to create gametophytic or sporophytic apomixis for application in agriculture.

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 [19]. 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
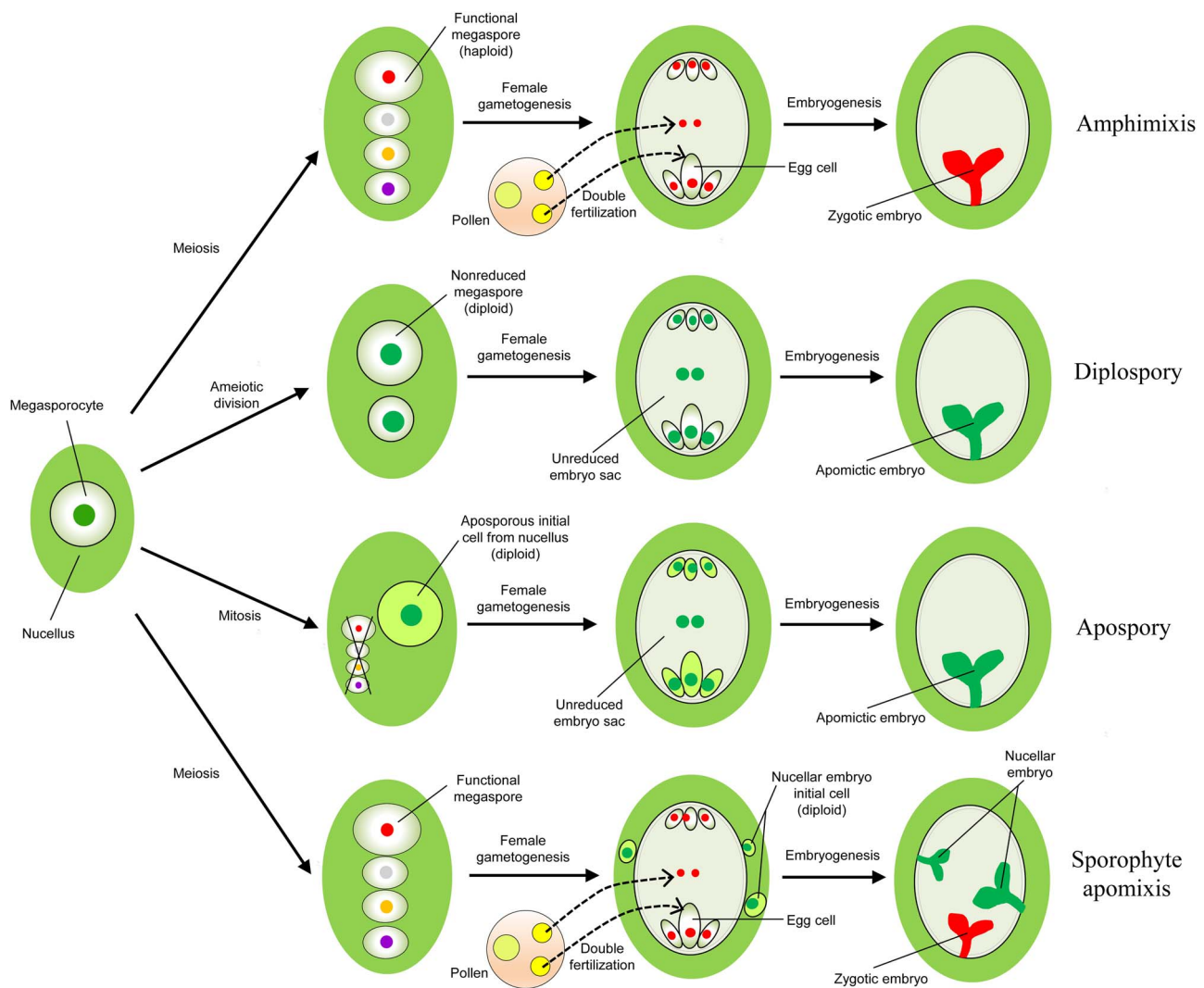
Figure 1. Schematic representation of sexual and apomictic embryo formation. In the process of amphimixis, the megaspore mother cell (MMC) undergoes mitosis and meiosis and develops into a seven-celled, eight-nucleate embryo sac, and then produces a zygotic embryo after double fertilization. For diplospory, the MMC undergoes ameiotic division and divides into two non-reduced megaspores. One of the non-reduced megaspores develops into an unreduced embryo sac. Then the diploid egg cell can directly develop into a parthenogenetic embryo. For apospory, the apospory initial cell from the nucellus forms an unreduced embryo sac and eventually develops into an asexual embryo. For sporophyte apomixis, at the same time as normal amphimixis, the nucellus-derived nucellar embryo initiation cells divide rapidly and enter the embryo sac, forming one or more nucellar embryos, which can coexist with the zygotic embryo.

provides nutrients for embryo development (Fig. 1). 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 [20, 21].

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 (Fig. 1). Based on the origin of the embryo, apomixis can be divided into two types, gametophytic apomixis and sporophytic apomixis (adventitious embryo) [1]. 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 [22, 23]. 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 [24]. 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 partheno-genetic 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 [25, 26]. Gametophytic apomixis completely replaces

amphimixis, and is regarded as obligate apomixis [23], while in most apomictic plants both sexual and asexual reproduction processes occur simultaneously in the same ovule, which is termed facultative apomixis [27]. In both diplosporous and aposporous ovules, the endosperm can develop spontaneously without fertilization or through pseudofertilization, providing nutrients for the development of the embryo [19].

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 [28]. 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 [29]. Multiple adventitious embryos can initiate in an individual ovule (Fig. 1).

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

Table 1. Information on candidate genes related to apomixis

Component of apomixis	Gene	Description	Genus	References
Apomeiosis	*APOLLO*	*APOLLO* is associated with egg cell formation in apomicts. It is highly expressed in apomictic ovules.	*Boechera*	64, 65
	UPGRADE2	*UPGRADE2* represents a long non-coding RNA and its expression is related to the formation of unreduced pollen.	*Boechera*	66
	AGO104	*AGO104* is involved in chromatin condensation during meiosis. Mutation of *AGO104* can produce an apomixis-like phenotype, producing functional unreduced female gametes.	*Tripsacum*	67
	PAIR1	PAIR1 protein is essential for homologous chromosome pairing in early meiotic prophase in rice.	*Oryza*	68
	ago9	AGO9-dependent sRNA silencing is important for specification of cell fate and initiation of gametogenesis in the *Arabidopsis* ovule.	*Arabidopsis*	30
	swi1	SWI1 encodes an unknown protein that is important for sister chromatid cohesion in the meiosis process.	*Arabidopsis*	31
	spo11-1/2	SPO11-1 and SPO11-2 encode Topo VIA proteins, which can induce meiotic double-strand break (DSB), which is required for meiotic recombination.	*Arabidopsis*	32,33
	mtopVIB	*MTOPVIB* encodes Topo VIB protein, which can interact with Topo VIA proteins to promote meiotic DSB formation.	*Arabidopsis*	34
	dfo	*DFO* is involved in DSB formation. Mutation of *DFO* severely affected homolog synapsis and recombination during meiosis.	*Arabidopsis*	35
	prd1	PRD1 participates in meiotic recombination and is required for meiotic DSB formation.	*Arabidopsis*	36
	rad50	Rad50 protein is required for telomere maintenance. Mutation of Rad50 will stimulate chromosomal recombination.	*Arabidopsis*	37,38
	dmc1	*DMC1* is involved in meiotic recombination. Mutants of *DMC1* exhibit defects in meiotic DSB formation.	*Arabidopsis*	39
	msh4	MSH4 is involved in crossover formation at the early step of recombination.	*Arabidopsis*	40
	asy1	ASY1 plays an essential role in homologous chromosome synapsis.	*Arabidopsis*	41
	rec8	Cohesin Rec8 plays an important role in reductional chromosome segregation.	*Arabidopsis*	42
	scc3	SCC3 protein is essential for the maintenance of centromere cohesion.	*Arabidopsis*	43
	ahp2	AHP2 is involved in bivalent formation and homologous chromosome segregation.	*Arabidopsis*	44
	osd1	OSD1 mutants cannot go into the second meiotic division.	*Arabidopsis*	45
	tam	TAM encodes an A-type cyclin that is involved in both meiosis I and meiosis II.	*Arabidopsis*	46,47
	tdm1	TDM1 is essential for meiotic termination after meiosis II.	*Arabidopsis*	48
Apospory	*QGJ*	*QGJ* is involved in the development of non-reduced embryo sacs in apomictic plants.	*Paspalum*	69
	GID1	Ectopic expression of *GID1* leads to the occurrence of MMC-like cells in the nucellus that do not have MMC identity.	*Brachiaria*	70
	DEFICIENSH	*DEFICIENSH* may be related to cellular differentiation of the MMC and megagametogenesis.	*Hieracium*	71
	PnTgs1-like	*PnTgs1-like* probably determines the fate of nucellar cells, as its reduced expression is associated with initiation of the apomictic pathway.	*Paspalum*	72
	SERK	Activation of *SERK* in nucellar cells can induce formation of the aposporous initial cell and development of the asexual embryo sac.	*Poa*	73
Endosperm development	*ORC3*	Defective *ORC3* mutants exhibit a normal female gametophyte but development of the embryo and endosperm is abolished.	*Paspalum*	74
	FIE	Expression of *FIE* is negatively correlated with parthenogenesis capacity. Mutant *FIE* allows endosperm development without fertilization.	*Malus, Arabidopsis*	51,75,76
	fis	*FIS* controls seed development after double fertilization. In *fis* mutants, partial development of seeds can occur without pollination.	*Arabidopsis*	52

(Continued)

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].

Table 1. Continued

Component of apomixis	Gene	Description	Genus	References
Parthenogenesis	ASGR-BBML	*ASGR-BBML* is expressed in unfertilized egg cells of apomictic *Pennisetum squamulatum* and activation of its expression in sexual pearl millet can also trigger parthenogenesis.	*Pennisetum, Cenchrus*	12,77
	PAR	The dominant *PAR* allele of dandelion is specifically expressed in egg cells and can trigger embryogenesis without fertilization.	*Taraxacum*	78
	MTL1	*MTL1* encodes a pollen-specific phospholipase that is involved in fertilization. Mutation of the *MTL1* gene can induce haploid formation in maize.	*Zea*	79
	msi1	The *MSI1* gene functions in chromatin assembly. Mutants of *MSI1* can produce parthenogenetic embryos.	*Arabidopsis*	49
	cenh3	Alteration of centromere-specific histone CENH3 can induce genome elimination and haploid formation.	*Arabidopsis*	50
Adventitious embryogenesis	RWP	The *CitRWP* gene co-segregates with the citrus nucellar embryo and is preferentially expressed in nucellar embryo initiation cells. Loss of *CitRWP* function can abolish nucellar embryogenesis in citrus.	*Citrus*	80,81
	AGL11	*AGL11* is a MADS-box transcription factor and is preferentially expressed at the apomictic nucellar embryo stage in *Zanthoxylum bungeanum*. Ectopic expression of *ZbAGL11* can lead to abnormal flower development and induce apomixis-like phenotypes in *Arabidopsis*.	*Zanthoxylum*	82

Miniature inverted-repeat transposable element transposons mediate activation of apomixis-controlling genes

Transposon insertions can affect the expression and function of adjacent genes and can cause phenotypic changes in plants [83–85]. With the development of research on apomixis, some evidence suggests that transposons may be involved in apomixis. In both aposporous *Cenchrus ciliaris* and *P. squamulatum*, the apospory-specific genomic region (*ASGR*) is located on a single chromosome that contains transposons and repeated sequences [9, 86, 87]. The hemizygous chromosomal region containing the *LOSS OF APOMEIOSIS* (*LOA*) locus in *Hieracium* also has abundant complex repeats and transposon sequences [88]. These structural features of the apomixis loci suggest that transposons might take part the induction or maintenance of apospory in these plants. Notably, our previous genetic analysis identified a miniature inverted-repeat transposable element (MITE) transposon insertion in the promoter region of the candidate gene (*CitRWP*) controlling sporophytic apomixis in *Citrus* [80]. This MITE transposon showed complete co-segregation with the polyembryony trait of *Citrus* in both natural and segregating populations. In polyembryonic citrus varieties, the *CitRWP* gene with a MITE transposon insertion is highly expressed. While in monoembryonic varieties, no MITE transposon insertion was found in the promoter region of the *CitRWP* gene and its expression was barely detectable. All these results suggest that the MITE transposon insertion in the promoter region of the *CitRWP* gene is required to enable sporophytic apomixis in citrus (Fig. 2A). Similarly, in apomictic dandelion (*Taraxacum officinale*) and hawkweed (*Hieracium piloselloides*) MITE transposons also exist in the upstream region of the parthenogenesis gene (*PAR*)

[78]. The MITE-containing promoter from dandelion can activate the *PAR*-homologous gene from sexual lettuce to reproduce the dandelion parthenogenetic phenotype, suggesting the decisive effect of the MITE transposon on parthenogenesis (Fig. 2B).

In both *Citrus* and dandelion, the MITE transposons inserted in the promoter region may be associated with upregulation of the adjacent genes, thereby controlling apomixis. It is likely that MITE insertions in the promoter of *CitRWP* or *PAR* genes lead to a transition from sexual reproduction to adventitious or parthenogenetic embryo development. Generally, MITE insertion in the promoter may impact gene expression through two different mechanisms: (i) by introducing a spatiotemporally specific activating element within the MITE; and (ii) by disrupting a repressive regulatory element that normally represses adjacent gene expression. Another mechanism influencing alteration of DNA methylation patterns should also be considered. Recently, a DNA methylome analysis revealed hypermethylation in the promoter of *CitRWP* in polyembryonic citrus, which contains a MITE insertion, while hypomethylation was detected in the promoter of *CitRWP* in monoembryonic citrus without a MITE insertion [89]. This result suggests that the MITE insertion may be related to the hypermethylation of *CitRWP*, which might activate gene transcription and further enable cells in the ovules of polyembryonic citrus to switch to an apomictic pathway.

Epigenetic regulation of apomixis

The initiation of apomixis is believed to be attributable to the downregulation of important genes in sexual reproduction, and epigenetic regulation enables reversible conversion between the two reproductive modes in plants. Transcriptome comparison of apomictic *Boechera*,
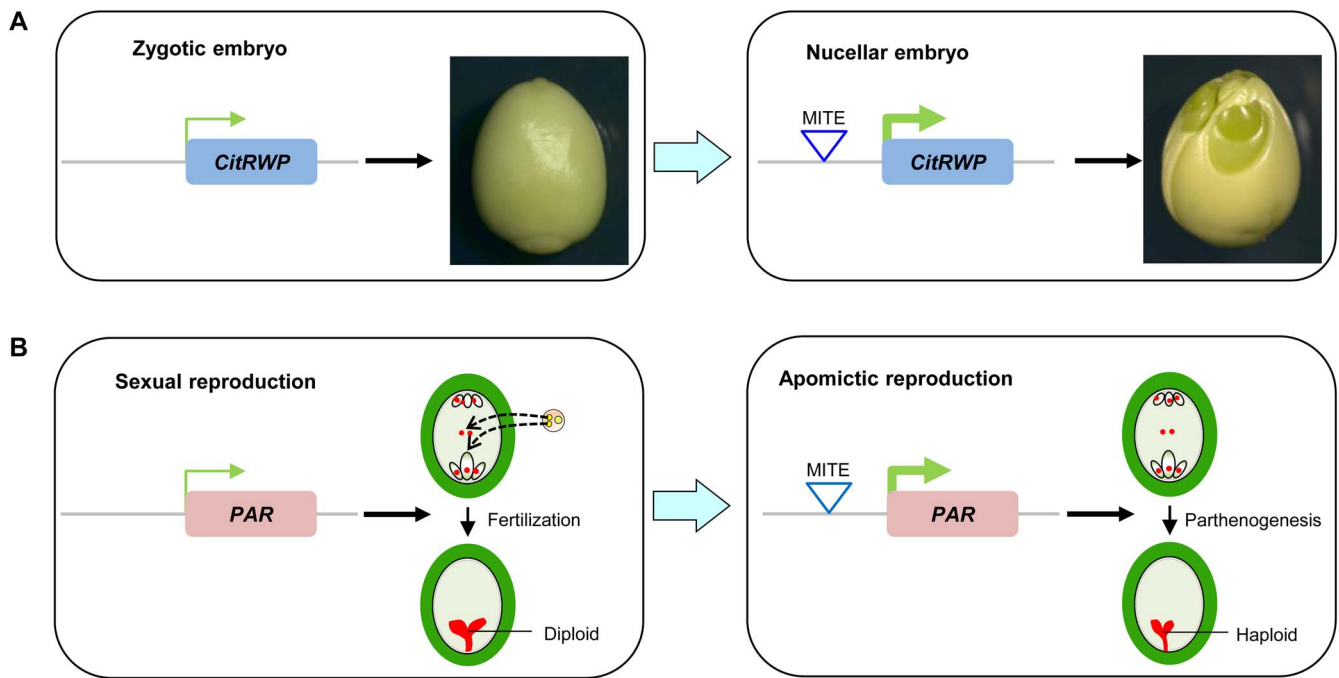
Figure 2. Typical cases of apomixis induced by MITE transposons. (A) A MITE transposon inserted in the *CitRWP* promoter activates gene expression, leading to multiple nucellar embryos in one seed of the polyembryonic citrus. In monoembryonic citrus there are no MITE transposon inserts in the *CitRWP* promoter and the gene is weakly expressed. (B) In sexual dandelions the *PAR* allele from the female parent with no MITE transposon insertion is not expressed in the egg cell, and the sexual diploid embryo comes from double fertilization. In polyploid apomicts the *PAR* allele with a MITE transposon insertion in its promoter is activated in the egg cell. Then the egg cell can directly develop into a haploid embryo without fertilization; this is parthenogenesis.

Hieracium, and *Hypericum* with related sexual lines revealed changes in siRNA synthesis and RNA-directed DNA methylation (RdDM)-related gene expression [90–92]. DNA methylation analysis showed that the overall methylation level of gametophyte-apomictic *C. ciliaris* [93] and sporophyte-apomictic citrus [89] was lower than that of sexually reproducing plants, suggesting that hypomethylation is related to apomictic reproduction, while in the gametophyte-apomictic *Paspalum* treatment with the DNA methylation inhibitor 5-azacytidine significantly reduced parthenogenesis frequency, suggesting that a high DNA methylation level may maintain apomictic reproduction [94].

The absence of important epigenetic pathway genes in model plants can lead to a phenotype resembling apomixis. Multiple mutants involved in siRNA synthesis and the RdDM pathway can form additional gametophytic cells, similar to what occurs in diplospory, such as *ago9*, *sgs3*, *rdr2*, *rdr6*, and *dcl3* [95]. Recent studies have shown that *AGO9* and *RDR6* gene mutations lead to ectopic expression of *SPL/NZZ*, which controls the differentiation of MMC cells, resulting in multiple MMC-like cells in the ovule [96].

Genetic events lead to apomixis: polyploidization and hybridization

Hybridization and polyploidization can widely activate transposable elements that are silenced by epigenetic modifications [83, 97]. Most gametophytic apomicts are

polyploid, and a causal relationship has been proposed between apomixis and polyploidization [6]. With respect to the cytological mechanism of apomixis, there is really a link between apomixis and polyploidization, as synthetic induction of polyploidy can induce apomixis from sexual plants [98, 99]. Nevertheless, apomixis can also occur in diploid plants [100, 101], suggesting that polyploidy is not a prerequisite of apomixis. The causal relationship between apomixis and polyploidization remains unclear. The point of view has been put forward that a polyploid genome can promote the optimum expression of apomixis [99], while it is also proposed that polyploidization may be a result of apomixis, which confers genomic stability. During the apomixis process, apomeiosis and parthenogenesis may increase the frequency of polyploidization [102, 103].

Most apomictic polyploids are allopolyploids, formed by hybridization between genetically divergent diploid species [104]. So it is also speculated that hybridization, rather than polyploidy, leads to apomixis [27, 105]. In genome-duplicated hybrids, asynchronous expression of the two sets of genes involved in female reproduction may result in precocious embryo sac initiation and embryogenesis [6]. In *Boechera*, diploid apomicts show high heterozygosity caused by the conjunction of disparate genomes, which suggests that the genomic consequences of hybridization may be related to gametophytic apomixis in this genus [106]. A hybrid origin of apomixis had also been proposed in the *Ranunculus cassubicus* complex, and some unique alleles that resulted from genomic

reorganization in allopolyploids might trigger apomixis [107]. Additionally, some sporophytic apomictic species, such as *Citrus* [108], *Mangifera* [109], and *Zanthoxylum* [110], all have a high level of heterozygosity.

Perspectives for future study and applications of apomixis in breeding

Apomixis is a fascinating phenomenon with great application potential for agriculture. Although increasing numbers of genes associated with apomixis have been identified, the gene regulatory network and molecular mechanisms of apomixis are not clear. Based on the candidate genes currently identified by genetic analysis, further studies are needed to dissect the upstream and downstream regulators. And a clear regulatory network of each component of apomixis will provide a strong foundation for the engineering of apomixis in crops. As apomixis occurs randomly in some genera of angiosperms and all steps of the apomixis process have evolved several times independently, exploring the origin and evolution of apomixis may provide more clues about the mechanism of apomixis. For example, the *CitRWP* gene has been proved to control nucellar embryony in *Citrus*, but not in its related genera in Rutaceae, including *Zanthoxylum*, *Murraya*, and *Poncirus*, although these genera exhibit a form of sporophytic apomixis similar to that of *Citrus* [111]. It is probable that all the genera in Rutaceae undergo the same apomixis pathway, but the mutations associated with apomixis in different genera may have occurred at different nodes of the pathway. Thus, identification of the different mutations leading to nucellar embryogenesis in each genus may contribute to deeper understanding of the regulatory pathways of sporophytic apomixis in Rutaceae.

Artificial creation of apomixis in crops is an effective way to fix heterosis and the ultimate goal of studying apomictic reproductive traits. Several studies have reported the engineering of gametophytic apomixis. The *MiMe* (substitute mitosis for meiosis) system was first created in *Arabidopsis* by simultaneous mutation of three key meiotic genes (*SPO11-1*, *REC8*, and *OSD1*) [112]. Hybridization of a *cenh3* null mutant expressing altered CENH3 protein, which can induce centromere-mediated genome elimination, with *MiMe* plants produced clonal reproduction though seeds [113]. In rice, triple mutations of three key meiotic genes (*PAIR1*, *REC8*, and *OSD1*) can also turn mitosis to meiosis (*MiMe*) [114]. Multiplex editing of the three key meiotic genes and the *MTL* gene resulted in plants that can propagate clonally through seeds [115]. Moreover, *MiMe* combined with the expression of a parthenogenesis gene, *BBM1*, in the egg cell can also induce clonal progeny of hybrid rice that retain genome-wide parental heterozygosity [116]. However, there are still some limitations in the application of the above synthetic apomixis strategies. The *MiMe* system combined with *CENH3* system depends on hybrid pollination [113], which restricted the commercial production of clonal seeds. In both the *MiMe* combined with *MTL1* system and the *MiMe* combined with *BBM1* system, the clonal seeds exhibited relatively low fertility, possibly due to the low frequency of parthenogenesis [115, 116]. Current apomixis strategies might be improved by increasing parthenogenesis induction efficiency. In addition, the *MiMe* combined with *BBM1* system requires self-pollination to initiate endosperm development and thus sexual seeds are also produced together with the clonal seeds. This system could potentially be improved by integrating genes that can promote autonomous endosperm development, such as *ORC* [74] and *FIE* [51].

As the mechanisms of sporophytic apomixis are less studied, application of sporophytic apomixis has been difficult. The *CitRWP* gene identified in citrus is a potential candidate to create sporophytic apomictic crops [80]. RWP-RK domain-containing (RKD) genes play important roles in the maintenance of egg-cell identity and their ectopic expression can promote somatic embryogenesis in *Arabidopsis* [117]. The specific expression of *CitRWP* in citrus ovules may enable the nucellar cells to acquire an embryonic fate. Additionally, a C2H2 zinc-finger domain-containing transcription factor gene (*CitZFP*), which is homologous to the dandelion parthenogenesis gene (*PAR*), is specifically expressed in apomictic cells [89]. This gene may be another candidate gene for engineering sporophytic apomixis. Further studies on the function and regulation of *CitRWP* and *CitZFP* genes in citrus are necessary for the utilization of sporophytic apomixis in apomixis breeding.

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Author contributions

Q.X. and Y.X. planned the outline of the review. Y.X. completed the first draft of the paper. H.J. and C.T. helped with literature collection and discussion. Q.X., X.W., and X.D. revised the paper. All authors approved the final paper.

Conflict of interest

The authors declare no competing interests.

References

1. Ozias-Akins P. Apomixis: developmental characteristics and genetics. *Crit Rev Plant Sci*. 2007;**25**:199–214.
2. Ozias-Akins P, van Dijk PJ. Mendelian genetics of apomixis in plants. *Annu Rev Genet*. 2007;**41**:509–37.

3. Spillane C, Curtis MD, Grossniklaus U. Apomixis technology development – virgin births in farmers' fields? *Nat Biotechnol*. 2004;**22**:687–91.

4. Calzada JPV, Crane CF, Stelly DM. Apomixis – the asexual revolution. *Science*. 1996;**274**:1322–3.

5. Smith J. Notice of a plant which produces seeds without any apparent action of pollen. *Transactions of the Linnaean Society of London* 1841;**18**:509–12.

6. Carman JG. Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispory, tetraspory, and polyembryony. *Biol J Linn Soc*. 1997;**61**:51–94.

7. Tas IC, Van Dijk PJ. Crosses between sexual and apomictic dandelions (*Taraxacum*). I. The inheritance of apomixis. *Heredity*. 1999;**83**:707–14.

8. Tucker MR, Araujo ACG, Paech NA *et al*. Sexual and apomictic reproduction in *Hieracium* subgenus *Pilosella* are closely interrelated developmental pathways. *Plant Cell*. 2003;**15**: 1524–37.

9. Akiyama Y, Hanna WW, Ozias-Akins P. High-resolution physical mapping reveals that the apospory-specific genomic region (ASGR) in *Cenchrus ciliaris* is located on a heterochromatic and hemizygous region of a single chromosome. *Theor Appl Genet*. 2005;**111**:1042–51.

10. Kotani Y, Henderson ST, Suzuki G *et al*. The LOSS OF APOMEIO-SIS (LOA) locus in *Hieracium praealtum* can function independently of the associated large-scale repetitive chromosomal structure. *New Phytol*. 2014;**201**:973–81.

11. Van Dijk PJ, Op den Camp R, Schauer SE. Genetic dissection of apomixis in dandelions identifies a dominant parthenogenesis locus and highlights the complexity of autonomous endosperm formation. *Genes*. 2020;**11**:961.

12. Conner JA, Mookkan M, Huo H *et al*. A parthenogenesis gene of apomict origin elicits embryo formation from unfertilized eggs in a sexual plant. *Proc Natl Acad Sci USA*. 2015;**112**: 11205–10.

13. Wakana A, Uemoto S, Adventive embryogenesis in Citrus. I. The occurrence of adventive embryos without pollination or fertilization. *Am J Bot*. 1987;**74**:517–30.

14. Liu DD, Fang MJ, Dong QL *et al*. Unreduced embryo sacs escape fertilization via a 'female-late-on-date' strategy to produce clonal seeds in apomictic crabapples. *Sci Hortic*. 2014;**167**:76–83.

15. Wu GL, Chen YH, Zhang PF *et al*. Apomixis and new selections of walnut. *Acta Hortic*. 2007;**760**:541–8.

16. Aron Y, Czosnek H, Gazit S *et al*. Polyembryony in mango (*Mangifera indica* L.) is controlled by a single dominant gene. *HortScience*. 1998;**33**:1241–2.

17. Beurton C. Gynoecium and perianth in *Zanthoxylum* s.l. (Rutaceae). *Plant Syst Evol*. 1994;**189**:165–91.

18. Kojima A, Nagato Y. Discovery of highly apomictic and highly amphimictic dihaploids in *Allium tuberosum*. *Sex Plant Reprod*. 1997;**10**:8–12.

19. Tucker MR, Koltunow AM. Sexual and asexual (apomictic) seed development in flowering plants: molecular, morphological and evolutionary relationships. *Funct Plant Biol*. 2009;**36**: 490–504.

20. Li DX, Chen SJ, Tian HQ. Advances in the study of zygote activation in higher plants. *Zygote*. 2021;**29**:12–9.

21. Wang K, Chen H, Ortega-Perez M *et al*. Independent parental contributions initiate zygote polarization in *Arabidopsis thaliana*. *Curr Biol*. 2021;**31**:4810–4816.e5.

22. Koltunow AM, Bicknell RA, Chaudhury AM. Apomixis: molecular strategies for the generation of genetically identical seeds without fertilization. *Plant Physiol*. 1995;**108**:1345–52.

23. Bicknell RA, Koltunow AM. Understanding apomixis: recent advances and remaining conundrums. *Plant Cell*. 2004; **16**:S228–45.

24. Schmidt A. Controlling apomixis: shared features and distinct characteristics of gene regulation. *Genes*. 2020;**11**:329.

25. Tucker MR, Paech NA, Willemse MT *et al*. Dynamics of callose deposition and β-1,3-glucanase expression during reproductive events in sexual and apomictic *Hieracium*. *Planta*. 2001;**212**: 487–98.

26. Wen XS, Ye XL, Li YQ *et al*. Embryological studies on apomixis in *Pennisetum squamulatum*. *J Integr Plant Biol*. 1998;**40**:598–604.

27. Koltunow AM, Grossniklaus U. Apomixis: a developmental perspective. *Annu Rev Plant Biol*. 2003;**54**:547–74.

28. Hojsgaard D, Horandl E. The rise of apomixis in natural plant populations. *Front Plant Sci*. 2019;**10**:358.

29. Koltunow AM, Soltys K, Nito N *et al*. Anther, ovule, seed, and nucellar embryo development in *Citrus sinensis* cv Valencia. *Can J Bot*. 1995;**73**:1567–82.

30. Olmedo-Monfil V, Durán-Figueroa N, Arteaga-Vázquez M *et al*. Control of female gamete formation by a small RNA pathway in *Arabidopsis*. *Nature*. 2010;**464**:628–32.

31. Boateng KA, Yang X, Dong F *et al*. SWI1 is required for meiotic chromosome remodeling events. *Mol Plant*. 2008;**1**:620–33.

32. Grelon M, Vezon D, Gendrot G *et al*. AtSPO11-1 is necessary for efficient meiotic recombination in plants. *EMBO J*. 2001;**20**: 589–600.

33. Hartung F, Wurz-Wildersinn R, Fuchs J *et al*. The catalytically active tyrosine residues of both SPO11-1 and SPO11-2 are required for meiotic double-strand break induction in *Arabidopsis*. *Plant Cell*. 2007;**19**:3090–9.

34. Vrielynck N, Chambon A, Vezon D *et al*. A DNA topoisomerase VI-like complex initiates meiotic recombination. *Science*. 2016;**351**:939–43.

35. Zhang C, Song Y, Cheng ZH *et al*. The *Arabidopsis thaliana* DSB formation (*AtDFO*) gene is required for meiotic double-strand break formation. *Plant J*. 2012;**72**:271–81.

36. De Muyt A, Vezon D, Gendrot G *et al*. AtPRD1 is required for meiotic double strand break formation in *Arabidopsis thaliana*. *EMBO J*. 2007;**26**:4126–37.

37. Gherbi H, Gallego ME, Jalut N *et al*. Homologous recombination *in planta* is stimulated in the absence of Rad50. *EMBO Rep*. 2001;**2**:287–91.

38. Vannier JB, Depeiges A, White C *et al*. Two roles for Rad50 in telomere maintenance. *EMBO J*. 2006;**25**:4577–85.

39. Couteau F, Belzile F, Horlow C *et al*. Random chromosome segregation without meiotic arrest in both male and female meiocytes of a *dmc1* mutant of *Arabidopsis*. *Plant Cell*. 1999;**11**: 1623–34.

40. Higgins JD, Armstrong SJ, Franklin FC *et al*. The *Arabidopsis MutS* homolog AtMSH4 functions at an early step in recombination: evidence for two classes of recombination in *Arabidopsis*. *Genes Dev*. 2004;**18**:2557–70.

41. Caryl AP, Armstrong SJ, Jones GH *et al*. A homologue of the yeast *HOP1* gene is inactivated in the *Arabidopsis* meiotic mutant *asy1*. *Chromosoma*. 2000;**109**:62–71.

42. Watanabe Y, Nurse P. Cohesin Rec8 is required for reductional chromosome segregation at meiosis. *Nature*. 1999;**400**:461–4.

43. Chelysheva L, Diallo Ś, Vezon D *et al*. AtREC8 and AtSCC3 are essential to the monopolar orientation of the kinetochores during meiosis. *J Cell Sci*. 2005;**118**:4621–32.

44. Schommer C, Beven A, Lawrenson T *et al*. AHP2 is required for bivalent formation and for segregation of homologous chromosomes in *Arabidopsis* meiosis. *Plant J*. 2003;**36**:1–11.

45. Cromer L, Heyman J, Touati S et al. OSD1 promotes meiotic progression via APC/C inhibition and forms a regulatory network with TDM and CYCA1;2/TAM. *PLoS Genet*. 2012;**8**: e1002865.

46. Magnard JL, Yang M, Chen YCS et al. The *Arabidopsis* gene *tardy asynchronous meiosis* is required for the normal pace and synchrony of cell division during male meiosis. *Plant Physiol*. 2001;**127**:1157–66.

47. Wang Y, Magnard JL, McCormick S et al. Progression through meiosis I and meiosis II in *Arabidopsis* anthers is regulated by an A-type cyclin predominately expressed in prophase I. *Plant Physiol*. 2004;**136**:4127–35.

48. Cifuentes M, Jolivet S, Cromer L et al. TDM1 regulation determines the number of meiotic divisions. *PLoS Genet*. 2016;**12**:e1005856.

49. Guitton AE, Berger F. Loss of function of MULTICOPY SUPPRESSOR OF IRA 1 produces nonviable parthenogenetic embryos in *Arabidopsis*. *Curr Biol*. 2005;**15**:750–4.

50. Ravi M, Chan SW. Haploid plants produced by centromere-mediated genome elimination. *Nature*. 2010;**464**:615–8.

51. Ohad N, Yadegari R, Margossian L et al. Mutations in FIE, a WD polycomb group gene, allow endosperm development without fertilization. *Plant Cell*. 1999;**11**:407–15.

52. Chaudhury AM, Ming L, Miller C et al. Fertilization-independent seed development in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA*. 1997;**94**:4223–8.

53. Kepiro JL, Roose ML. AFLP markers closely linked to a major gene essential for nucellar embryony (apomixis) in *Citrus maxima* × *Poncirus trifoliata*. *Tree Genet Genomes*. 2009; **6**:1–11.

54. Vašut RJ, Vijverberg K, van Dijk PJ et al. Fluorescent in situ hybridization shows DIPLOSPOROUS located on one of the NOR chromosomes in apomictic dandelions (*Taraxacum*) in the absence of a large hemizygous chromosomal region. *Genome*. 2014;**57**:609–20.

55. Noyes RD, Rieseberg LH. Two independent loci control agamospermy (apomixis) in the triploid flowering plant *Erigeron annuus*. *Genetics*. 2000;**155**:379–90.

56. Schallau A, Arzenton F, Johnston AJ et al. Identification and genetic analysis of the APOSPORY locus in *Hypericum perforatum* L. *Plant J*. 2010;**62**:773–84.

57. Albertini E, Porceddu A, Ferranti F et al. Apospory and parthenogenesis may be uncoupled in *Poa pratensis*: a cytological investigation. *Sex Plant Reprod*. 2001;**14**:213–7.

58. Conner JA, Gunawan G, Ozias-Akins P. Recombination within the apospory specific genomic region leads to the uncoupling of apomixis components in *Cenchrus ciliaris*. *Planta*. 2013;**238**: 51–63.

59. Catanach AS, Erasmuson SK, Podivinsky E et al. Deletion mapping of genetic regions associated with apomixis in *Hieracium*. *Proc Natl Acad Sci USA*. 2006;**103**:18650–5.

60. Ogawa D, Johnson SD, Henderson ST et al. Genetic separation of autonomous endosperm formation (AutE) from the two other components of apomixis in *Hieracium*. *Plant Reprod*. 2013;**26**: 113–23.

61. Barcaccia G, Albertini E. Apomixis in plant reproduction: a novel perspective on an old dilemma. *Plant Reprod*. 2013;**26**: 159–79.

62. Hand ML, Koltunow AM. The genetic control of apomixis: asexual seed formation. *Genetics*. 2014;**197**:441–50.

63. Zappacosta D, Gallardo J, Carballo J et al. A high-density linkage map of the forage grass *Eragrostis curvula* and localization of the diplospory locus. *Front Plant Sci*. 2019;**10**:918.

64. Corral JM, Vogel H, Aliyu OM et al. A conserved apomixis-specific polymorphism is correlated with exclusive exonuclease expression in premeiotic ovules of apomictic *Boechera* species. *Plant Physiol*. 2013;**163**:1660–72.

65. Mau M, Lovell JT, Corral JM et al. Hybrid apomicts trapped in the ecological niches of their sexual ancestors. *Proc Natl Acad Sci USA*. 2015;**112**:2357–65.

66. Mau M, Corral JM, Vogel H et al. The conserved chimeric transcript UPGRADE2 is associated with unreduced pollen formation and is exclusively found in apomictic *Boechera* species. *Plant Physiol*. 2013;**163**:1640–59.

67. Singh M, Goel S, Meeley RB et al. Production of viable gametes without meiosis in maize deficient for an ARGONAUTE protein. *Plant Cell*. 2011;**23**:443–58.

68. Nonomura K, Nakano M, Fukuda T et al. The novel gene *HOMOLOGOUS PAIRING ABERRATION IN RICE MEIOSIS1* of rice encodes a putative coiled-coil protein required for homologous chromosome pairing in MEIOSIS. *Plant Cell*. 2004;**16**: 1008–20.

69. Mancini M, Permingeat H, Colono C et al. The MAP3K-coding *QUI-GON JINN* (*QGJ*) gene is essential to the formation of unreduced embryo sacs in *Paspalum*. *Front Plant Sci*. 2018; **9**:1547.

70. Ferreira LG, de Alencar Dusi DM, Irsigler AST et al. GID1 expression is associated with ovule development of sexual and apomictic plants. *Plant Cell Rep*. 2018;**37**:293–306.

71. Guerin J, Rossel JB, Robert S et al. A DEFICIENS homologue is down-regulated during apomictic initiation in ovules of *Hieracium*. *Planta*. 2000;**210**:914–20.

72. Siena LA, Ortiz JP, Leblanc O et al. PnTgs1-like expression during reproductive development supports a role for RNA methyltransferases in the aposporous pathway. *BMC Plant Biol*. 2014;**14**:297.

73. Albertini E, Marconi G, Reale L et al. SERK and APOSTART. Candidate genes for apomixis in *Poa pratensis*. *Plant Physiol*. 2005;**138**: 2185–99.

74. Siena LA, Ortiz JPA, Calderini O et al. An apomixis-linked *ORC3-like* pseudogene is associated with silencing of its functional homolog in apomictic *Paspalum simplex*. *J Exp Bot*. 2016;**67**: 1965–78.

75. Liu DD, Dong QL, Sun C et al. Functional characterization of an apple apomixis-related *MhFIE* gene in reproduction development. *Plant Sci*. 2012;**185–186**:105–11.

76. Liu DD, Dong QL, Fang MJ et al. Ectopic expression of an apple apomixis-related gene *MhFIE* induces co-suppression and results in abnormal vegetative and reproductive development in tomato. *J Plant Physiol*. 2012;**169**:1866–73.

77. Akiyama Y, Goel S, Conner JA et al. Evolution of the apomixis transmitting chromosome in *Pennisetum*. *BMC Evol Biol*. 2011;**11**:289.

78. Underwood CJ, Vijverberg K, Rigola D et al. A PARTHENOGENESIS allele from apomictic dandelion can induce egg cell division without fertilization in lettuce. *Nat Genet*. 2022;**54**:84–93.

79. Kelliher T, Starr D, Richbourg L et al. MATRILINEAL, a sperm-specific phospholipase, triggers maize haploid induction. *Nature*. 2017;**542**:105–9.

80. Wang X, Xu Y, Zhang S et al. Genomic analyses of primitive, wild and cultivated citrus provide insights into asexual reproduction. *Nat Genet*. 2017;**49**:765–72.

81. Shimada T, Endo T, Fujii H et al. MITE insertion-dependent expression of *CitRKD1* with a RWP-RK domain regulates somatic embryogenesis in citrus nucellar tissues. *BMC Plant Biol*. 2018;**18**:166.

82. Fei X, Shi Q, Qi Y *et al*. ZbAGL11, a class D MADS-box transcription factor of *Zanthoxylum bungeanum*, is involved in sporophytic apomixis. *Hortic Res*. 2021;**8**:23.

83. Chuong EB, Elde NC, Feschotte C. Regulatory activities of transposable elements: from conflicts to benefits. *Nat Rev Genet*. 2017;**18**:71–86.

84. Martin A, Troadec C, Boualem A *et al*. A transposon-induced epigenetic change leads to sex determination in melon. *Nature*. 2009;**461**:1135–8.

85. Ong-Abdullah M, Ordway JM, Jiang N *et al*. Loss of Karma transposon methylation underlies the mantled somaclonal variant of oil palm. *Nature*. 2015;**525**:533–7.

86. Akiyama Y, Conner JA, Goel S *et al*. High-resolution physical mapping in *Pennisetum squamulatum* reveals extensive chromosomal heteromorphism of the genomic region associated with apomixis. *Plant Physiol*. 2004;**134**:1733–41.

87. Conner JA, Goel S, Gunawan G *et al*. Sequence analysis of bacterial artificial chromosome clones from the apospory-specific genomic region of *Pennisetum* and *Cenchrus*. *Plant Physiol*. 2008;**147**:1396–411.

88. Okada T, Ito K, Johnson SD *et al*. Chromosomes carrying meiotic avoidance loci in three apomictic eudicot *Hieracium* subgenus *Pilosella* species share structural features with two monocot apomicts. *Plant Physiol*. 2011;**157**:1327–41.

89. Jia HH, Xu YT, Yin ZP *et al*. Transcriptomes and DNA methylomes in apomictic cells delineate nucellar embryogenesis initiation in citrus. *DNA Res*. 2021;**28**:dsab014.

90. Schmidt A, Schmid MW, Klostermeier UC *et al*. Apomictic and sexual germline development differ with respect to cell cycle, transcriptional, hormonal and epigenetic regulation. *PLoS Genet*. 2014;**10**:e1004476.

91. Rabiger DS, Taylor JM, Spriggs A *et al*. Generation of an integrated *Hieracium* genomic and transcriptomic resource enables exploration of small RNA pathways during apomixis initiation. *BMC Biol*. 2016;**14**:86.

92. Galla G, Zenoni S, Avesani L *et al*. Pistil transcriptome analysis to disclose genes and gene products related to aposporous apomixis in *Hypericum perforatum* L. *Front Plant Sci*. 2017;**8**:79.

93. Kumar S. Epigenetic control of apomixis: a new perspective of an old enigma. *Adv Plants Agric Res*. 2017;**7**:1–8.

94. Podio M, Cáceres ME, Samoluk SS *et al*. A methylation status analysis of the apomixis-specific region in *Paspalum* spp. suggests an epigenetic control of parthenogenesis. *J Exp Bot*. 2014;**65**:6411–24.

95. Hernández-Lagana E, Rodríguez-Leal D, Lúa J *et al*. A multigenic network of ARGONAUTE4 clade members controls early megaspore formation in *Arabidopsis*. *Genetics*. 2016;**204**: 1045–56.

96. Mendes MA, Petrella R, Cucinotta M *et al*. The RNA-dependent DNA methylation pathway is required to restrict SPOROCYTE-LESS/NOZZLE expression to specify a single female germ cell precursor in *Arabidopsis*. *Development*. 2020;**147**:dev194274.

97. Gantuz M, Morales A, Bertoldi MV *et al*. Hybridization and polyploidization effects on LTR-retrotransposon activation in potato genome. *J Plant Res*. 2022;**135**:81–92.

98. Quarin CL, Hanna WW. Effect of three ploidy levels on meiosis and mode of reproduction in *Paspalum hexastachyum*. *Crop Sci*. 1980;**20**:69–75.

99. Quarin CL, Espinoza F, Martinez EJ *et al*. A rise of ploidy level induces the expression of apomixis in *Paspalum notatum*. *Sex Plant Reprod*. 2001;**13**:243–9.

100. Bicknell RA. Isolation of a diploid, apomictic plant of *Hieracium aurantiacum*. *Sex Plant Reprod*. 1997;**10**:168–72.

101. Schranz ME, Dobes C, Koch MA *et al*. Sexual reproduction, hybridization, apomixis, and polyploidization in the genus *Boechera* (Brassicaceae). *Am J Bot*. 2005;**92**:1797–810.

102. Albertini E, Barcaccia G, Carman JG *et al*. Did apomixis evolve from sex or was it the other way around? *J Exp Bot*. 2019;**70**: 2951–64.

103. Hojsgaard D. Transient activation of apomixis in sexual neotriploids may retain genomically altered states and enhance polyploid establishment. *Front Plant Sci*. 2018; **9**:230.

104. Kearney M. Hybridization, glaciation and geographical parthenogenesis. *Trends Ecol Evol*. 2005;**20**:495–502.

105. Mogie M. *The Evolution of Asexual Reproduction in Plants*. London: Chapman & Hall; 1992.

106. Beck JB, Alexander PJ, Allphin L *et al*. Does hybridization drive the transition to asexuality in diploid *Boechera*? *Evolution*. 2012;**66**:985–95.

107. Paun O, Stuessy TF, Hörandl E. The role of hybridization, polyploidization and glaciation in the origin and evolution of the apomictic *Ranunculus cassubicus* complex. *New Phytol*. 2006;**171**: 223–36.

108. Wu GA, Terol J, Ibanez V *et al*. Genomics of the origin and evolution of *Citrus*. *Nature*. 2018;**554**:311–6.

109. Wang P, Luo Y, Huang J *et al*. The genome evolution and domestication of tropical fruit mango. *Genome Biol*. 2020; **21**:60.

110. Feng S, Liu Z, Hu Y *et al*. Genomic analysis reveals the genetic diversity, population structure, evolutionary history and relationships of Chinese pepper. *Hortic Res*. 2020;**7**:158.

111. Xu Y, Jia H, Wu X *et al*. Regulation of nucellar embryony, a mode of sporophytic apomixis in *Citrus* resembling somatic embryogenesis. *Curr Opin Plant Biol*. 2021;**59**:101984.

112. d'Erfurth I, Jolivet S, Froger N *et al*. Turning meiosis into mitosis. *PLoS Biol*. 2009;**7**:e1000124.

113. Marimuthu MP, Jolivet S, Ravi M *et al*. Synthetic clonal reproduction through seeds. *Science*. 2011;**331**:876.

114. Mieulet D, Jolivet S, Rivard M *et al*. Turning rice meiosis into mitosis. *Cell Res*. 2016;**26**:1242–54.

115. Wang C, Liu Q, Shen Y *et al*. Clonal seeds from hybrid rice by simultaneous genome engineering of meiosis and fertilization genes. *Nat Biotechnol*. 2019;**37**:283–6.

116. Khanday I, Skinner D, Yang B *et al*. A male-expressed rice embryogenic trigger redirected for asexual propagation through seeds. *Nature*. 2019;**565**:91–5.

117. Waki T, Hiki T, Watanabe R *et al*. The *Arabidopsis* RWP-RK protein RKD4 triggers gene expression and pattern formation in early embryogenesis. *Curr Biol*. 2011;**21**:1277–81.