REVIEW

The steps from sexual reproduction to apomixis

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Abstract

Main conclusion **In this paper, an interaction model of apomixis-related genes was constructed to analyze the emergence of apomictic types. It is speculated that apomixis technology will be first implemented in gramineous plants.**

Apomixis (asexual seed formation) is a phenomenon in which a plant bypasses the most fundamental aspects of sexual reproduction—meiosis and fertilization—to form a viable seed. Plants can form seeds without fertilization, and the seed genotype is consistent with the female parent. The development of apomictic technology would be revolutionary for agriculture and for food production as it would reduce costs and breeding times and also avoid many complications typical of sexual reproduction (e.g. incompatibility barriers) and of vegetative propagation (e.g. viral transfer). The application of apomictic reproductive technology has the potential to revolutionize crop breeding. This article reviews recent advances in apomixis in cytology and molecular biology. The general idea of identifying apomixis was proposed and the process of the emergence of non-fusion types was discussed. To better understand the apomixis mechanism, an apomixis regulatory model was established. At the same time, the realization of apomixis technology is proposed, which provides reference for the research and application of apomixis.

Keywords Apomixis · Genetic breeding · Apospory · Plant reproduction · Seed development · Embryonic development

Introduction

In angiosperms, double fertilization is the common reproductive pattern in which two sperms combine with an egg cell and a central cell. The former develops into an embryo, the embryonic form of a new plant, and the latter develops into an endosperm whose function is to support the early growth of this new plant (Lopes and Larkins 1993). During sexual reproduction, fertile pollen is essential to fertilize the central nucleus of the embryo sac and ensure viable seed production (Boldrini et al. 2006). By contrast, seed formation also occurs in apomictic plants but without fertilization. In apomictic plants, the genetic information of the offspring is consistent with the female parent, but the process of formation is different. Apomixis can be divided into three types: displospory, apospory, and adventitious embryony. Among them, displospory and apospory are derived from

unreduced embryo sacs, and adventitious embryony is derived from somatic cells (Koltunow 1993).

In agriculture, apomixis has the potential to transform plant breeding, allowing new varieties to retain valuable traits through asexual reproduction. The production of seeds without sexual union is considered the holy grail of plant biology (Hofmann 2010). Some families naturally show particularly high frequencies of apomixis, examples are: Poaceae (Sharma et al. 2014; Boldrini et al. 2006; Hojsgaard et al. 2008; Albertini et al. 2005; Niemann et al. 2012), Asteraceae (Whitton et al. 2008; Hand et al. 2015), Rosaceae (DOBEŠ et al. 2015; Burgess et al. 2014) and Melastomataceae (Mendes-Rodrigues and Oliveira 2012; Maia et al. 2016). In 1986, Richards counted known apomictic species, finding more than 300 taxa, distributed over 35 families (Richards 1986). Today, according to incomplete statistics, plants found to have apomictic reproductive ability exceed 400 genera (Grimanelli et al. 2003; Koltunow and Grossniklaus 2003).

However, the taxonomic distribution of apomictic taxa across the plant lineages is uneven, with estimations of 0.1% in angiosperms, up to 10% in ferns, and with little or no

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evidence of its existence in gymnosperms, mosses, liverworts or hornworts (Walker 1966; Lovis 1977; Asker 1992; Pichot 2001; Park 2003). Sexual reproduction is ancestral in homosporous ferns, and the transition to apomixis requires the evolution and alternation of two distinct phenomena: diplospory and apogamy. These represent the avoidance of meiotic reduction during sporogenesis (diplospory) and the spontaneous development of a sporophyte without fertilization (apogamy) (Lovis 1977). Apomictic 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 (Koltunow 1993; Nogler 1984). Most apomictic plants retain some potential for sexual reproduction, called facultative apomixis (Koltunow and Grossniklaus 2003). Apomixis leads to genetically identical offspring and this would allow a crop to retain complex features that are of value and also the production of seeds with superior traits, directly from the mother plant. Through these the seed production cycle would be shortened and the high cost of producing hybrid seeds would be eliminated (Bicknell and Koltunow 2004). Although apomixis is potentially important for crop breeding and has been studied extensively, the genetic regulation of apomixis remains unclear and the results obtained in studies of the apomictic mechanism are not always consistent (Galla et al. 2017). Over the last few years, reviews have focused on various aspects of apomixis including the different genetic control mechanisms. The study of apomixis from cytological and molecular biological perspectives may facilitate the application of this technology to key crops.

How to determine apomixis

To study the mechanism of apomixis, the first step is to find and identify which plants have apomictic characteristics. Plants can still produce fertile offspring after emasculation or blocking pollination, and it can be initially determined that the material studied is apomictic. In addition, the test of maintenance of maternal genotye in the offspring can be used to determine whether the progeny genes are all derived from the female parent, and the ploidy relationship between the endosperm and the somatic cells can be examined by flow cytometry to further prove that the material has apomictic characteristics. Microscopic techniques are used to observe the development of the embryo sac and to determine the source of the embryo to determine the type of apomixis. It is also possible to further determine whether the plant is obligate apomixis or facultative apomixis in combination with pollination experiments. Finally, apomixis can be identified at the molecular level, for example by looking

for specific genes or markers, *UPGRADE2* is highly upregulated in pollen mother cells, whereas in homologous reproduction there is no homologue in *Boechera* spp., which is identified as a specific transcript unique to apomixis (Mau et al. 2013). The emergence of specific sequences has made it possible to develop apomictic markers. Biomarkers provide an idea for the identification of apomictic, but it seems unrealistic to screen for universal marker genes in a wide range of species. It can be combined with other methods of identification to identify apomixis as an adjunct.

Cytological study of apomixis

There are two main forms of apomixis (Fig. 1): (1) sporophytic and (2) gametophytic. Sporophytic apomixis involves the direct formation of an embryo from a somatic cell in the ovule. In gametophytic apomixis the embryo sac is obtained via apospory and diplospory. In both cases, the unreduced egg cell develops parthenogenetically into an embryo. At the same time, endosperm development in apomictic species may be independent of fertilization (autonomous endosperm formation) or may need the fusion of the polar nuclei with a pollen nucleus (pseudogamy) (Koltunow and Grossniklaus 2003).

In facultative apomixis in sexual plants, the appearance and differentiation of archesporial cells occur at much the same time. During the development of the aposporous embryo sac, the nucellus cells close to the megaspores gradually become larger and form an aposporous initial. After two mitoses, these develop into an aposporous embryo sac. In a study of the ovule of *Poncirus trifoliate*, aposporous initials were not evident prior to megaspore formation but, in one ovary, an archesporial cell was observed with nearby enlarged nuclear cells—possibly representing an aposporous initial (Sharma et al. 2014).

Polyembryony is more common than might be expected in sexually reproducing species. In general, it is associated with apomixis (Mendes-Rodrigues and Oliveira 2012). The emergence of polyembryony is thought to be a result of apomixis. Polyembryony is the phenomenon of forming multiple embryos in one ovule. There are many species exhibiting polyembryonal behavior including *Mangifera indica* L. (Litz et al. 1982), *Citrus unshiu* (Nakano et al. 2012) and *Clidemia hirta* (L.) (Mendes-Rodrigues and Oliveira 2012). The occurrence of polyembryony is believed to be caused by apomixis and generally occurs through adventitious embryo development of nucellar cells. The existence of polyembryony in a species is usually associated with one in which there is a high probability of apomixis. Indeed, the presence of polyembryony is considered indicative of apomixis.
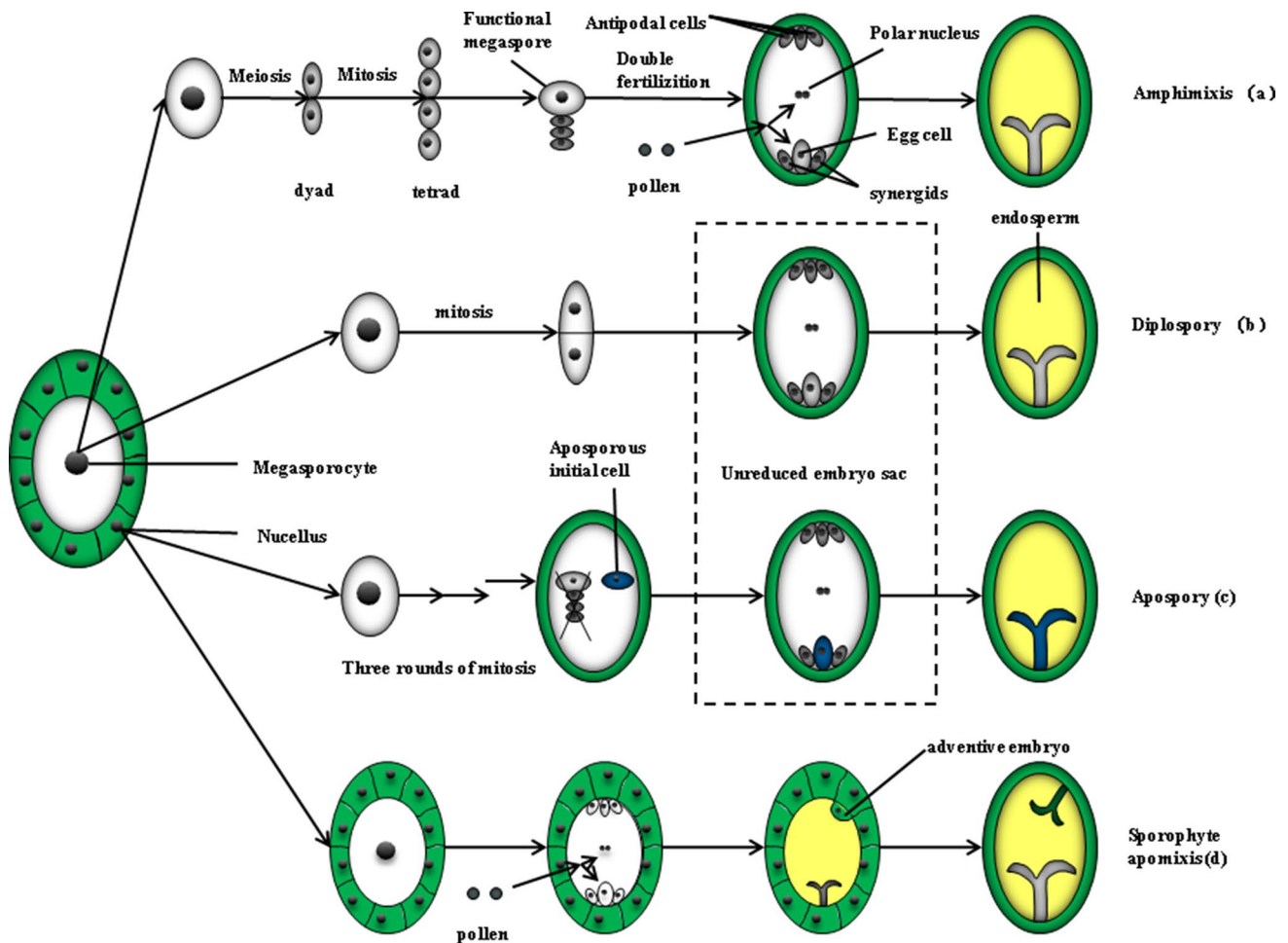
Fig. 1 Amphimictic and apomictic embryo formation. **a** Amphimixis, the megaspore mother cells undergo mitosis and meiosis to form an embryo sac with 8 nucleus, and then form embryos under the double fertilization; (**b**) Diplospory, the megaspore mother cells undergo mitosis to form unreduced embryo sac, and the apomictic initiation cells originate from the location of the megaspore mother cells and eventually develop into embryos; (**c**) Apospory, the aposporous initial cells (AI) near the megaspore mother cells form an unreduced embryo sac undergoes three rounds of mitosis and eventually develop into embryos; (**d**) Sporophyte apomixis, the apomictic initiation cells are derived from ovules, which rapidly divide and invade the embryo sac, producing one or more embryos, and sporophyte apomixis can coexist with amphimixis

Some apomictic species and their biological characteristics are summarized in Table 1, including Poaceae, Asteraceae, Rutaceae, Rosaceae, Melastomeae, Ranunculaceae and the like. According to the information available (Table 1), the majority of apomictic plant species are polyploid. Polyploidy is commonly accompanied by changes in the reproductive system such as the breakdown of self-incompatibility (Barrett 1988). This leads to instability of the reproductive system and this, in turn, to the emergence of new types of reproduction. The generation of apomictic characteristics is closely related to the environment in which plants live over long periods. In addition, facultative apomixis plants retain sexual reproduction patterns but will reproduce asexually in the absence of a male gamete or sexually after fertilization. These two modes can exist together and are independent (Vielle-Calzada et al. 1996). However, sexual reproduction and apomixis have been shown to have different regulatory pathways in cell cycle control, hormonal pathways, epigenetic and transcriptional regulation. For example, spermidine metabolism is involved in somatic embryogenesis in various plants and is a specific signal transduction pathway for the apomictic germline (Schmidt et al. 2014).

In the study of apomictic species, some still maintain an ability for gametogenesis, the facultative apomicts. As a biological trait, facultative apomixis has evolved over a very long period. It is a stable trait that allows rapid colonization of large areas. This situation provides genetic researchers with abundant plant material and with sufficient time for testing (Hojsgaard et al. 2014). The genus *Boechera* is closely related
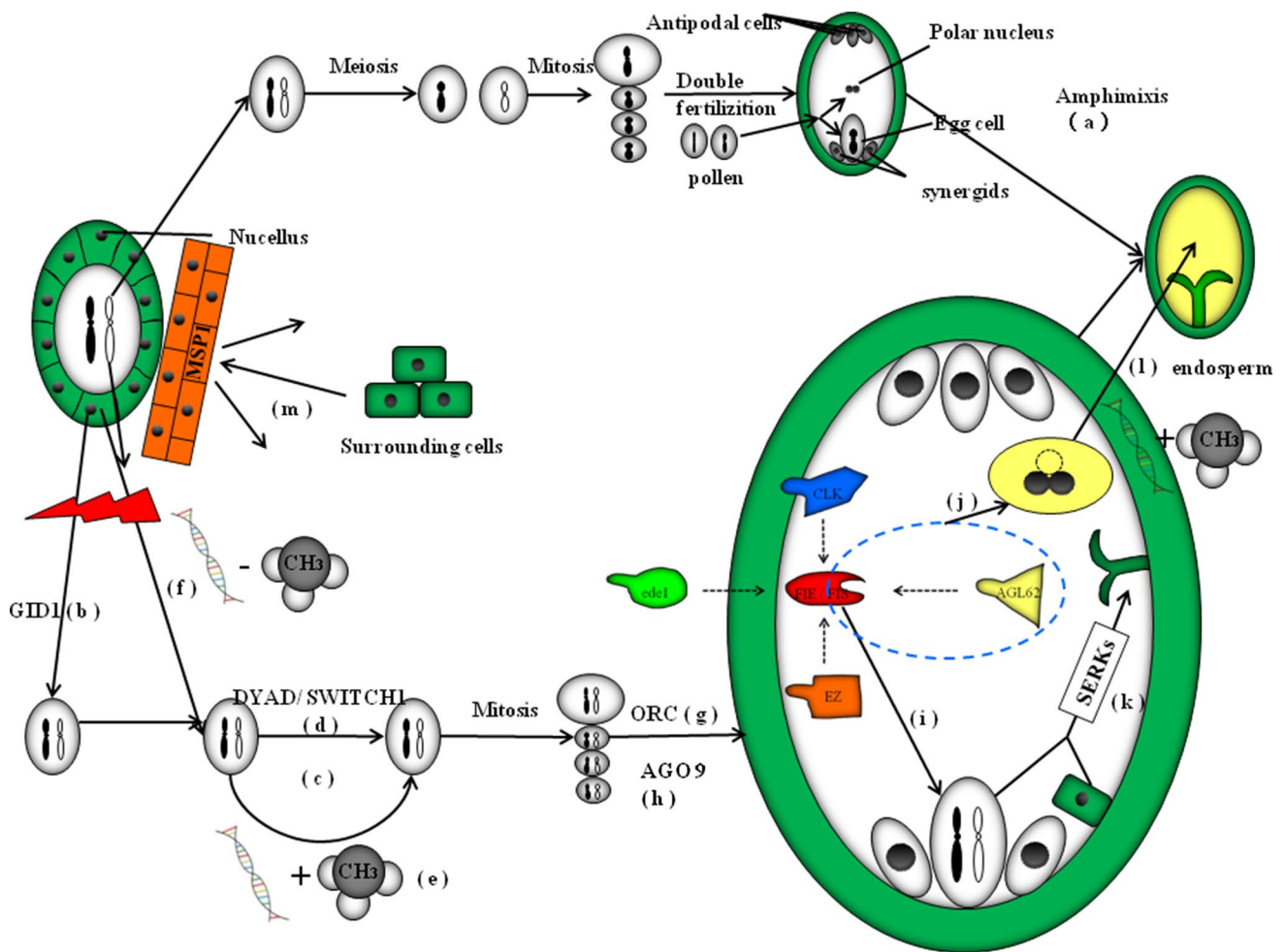
Fig. 2 Model of apomixis. **a** Amphimixis. **b** *GID1* overexpression triggers differentiation of MMC-like cells (Ferreira et al. 2018). **c** Mitosis or apomeiosis. **d** *DYAD / SWITCH1* gene is considered necessary in the process of female gamete meiosis. Its absence can cause meiosis of the megasporocytes to fail (Agashe et al. 2002; Boateng et al. 2008). **e** DNA methylation is often associated with apomeiosis, and methylation is always present during subsequent reproduction (Podio et al. 2014; Selva et al. 2017). **f** Demethylation can significantly inhibit the occurrence of parthenogenesis (Podio et al. 2014). **g** *ORC* is a multiprotein complex which controls DNA replication and cell differentiation in eukaryotes (Siena et al. 2016). **h** *ARGONAUTE 9* (*AGO9*) controls female gamete formation by limiting the specification of gametophyte precursors in a non-cell-autonomous manner (Olmedo–Monfil et al. 2010). **i** *FIE* genes, co-regulated with *CLK*, *ede1*, *EZ* and other related genes, can promote the independent development of egg cells into embryos without fertilization (Liu et al. 2012b, 2012a). **j** AGL62 binds to FIS to form a complex and mediates the cellulogenesis of the endosperm (Hehenberger et al. 2012). **k** SERKs family genes have been shown to be the switches of somatic cell differentiation, playing an active role in apomimetic reproduction (Albertini et al. 2005; Podio et al. 2014). **l** Modification of DNA methylation is one mechanism through which imprinting may be altered to allow endosperm development in apomicts (Spielman et al. 2003). **m** Surrounding cells can be blocked out of the nucellar cells by *MSP1*, a gene that controls early sporogenic development (Nonomura 2003)

to Arabidopsis, contains apomictic and sexual reproduction species, and has low ploidy levels. It is an ideal material for studying apomixis (Aliyu et al. 2010). Therefore, the extensive

molecular genetic resources that exist for *A. thaliana*—including the complete genome sequence and wide range of reproductive mutants—can be used to identify and isolate genes

Table 1 Apomictic species and related information

Family	Species	Ploidy	Type of apomixis	References
Poaceae	*Cenchrus ciliaris*	Tetraploid ($2n = 4x = 36$)	Facultative apomixis	Dwivedi et al. (2007)
	Paspalum minus	Diploid, tetraploid, and pentaploid ($2x$, $4\times$ and $5x$, $n = 10$)	Apomixis	Bonilla and Quarin (1997)
	Poa pratensis	Diploid ($2n = 42$)	Facultative apomixis	Niemann et al. (2012)
	Paspalum notatum	Tetraploid ($2n = 4x = 40$)	Obligate apomixis	Podio et al. (2014)
	Paspalum Anachyris	Tetraploid ($2n = 4x = 40$)	Apomixis	Hojsgaard et al. (2008)
	Pennisetum glaucum	Diploid ($2n = 2x = 14$)	Sexual or apomixis	Sahu et al. (2012)
	Brachiaria decumbens	Tetraploid $2n = 4x = 36$	Sexual or apomixis	Worthington et al. (2016)
	Brachiaria humidicola	Hexaploid $2n = 6x = 54$/ heptaploid ($2n = 7x = 42$)	Apomixis	Boldrini et al. (2006)
	Potentilla puberula	Tetraploid ($2n = 4x = 28$)	Preferentially apomictic	Dobes et al. (2013, 2018)
	Eragrostis curvula	Polyploid ($4\times$ to $7x$, $n = 10$)	Apomixis	Selva et al. (2012)
	Panicum maximum	Tetraploid $2n = 4x = 32$	Apomixis	Lu and Vasil (1981); Radhakrishna et al. (2018)
Asteraceae	*Hieracium praealtum*	Aneuploid ($3x + 4 = 2n = 31$), tetraploid ($3x = 2n = 27$)	Facultative apomixis	Okada et al. (2013)
	Hieracium pilosella	Tetraploid ($2n = 4x = 36$)	Specialist apomixis	Guerin et al. (2000)
	Erigeron annuus	Triploid (apomixis) or diploid (sexual) $2n = 3x = 27$, $2n = 2x = 18$	Facultative apomixis	Noyes and Rieseberg (2000)
	Crepis barbigera	Polyploid (~ 7–$8x$)	Apomixis	Whitton et al. (2008); Hersh et al. (2016)
	Taraxacum officinale	Triploid (apomixis) $2n = 3x = 24$, diploid sexuals ($2n = 2x = 16$)	Facultative apomixis	Dijk and Bakx-Schotman (2004)
Rutaceae	*Poncirus trifoliata*	Tetraploid($2n = 4x = 36$)	Facultative apomixis	Hussain et al. (2011)
	Citrus reticulata	Diploid ($2n = 2x = 18$)	Facultative apomixis	Wang et al. (2017)
Rosaceae	*Amelanchier*	Triploid ($2n = 3x = 51$)	Apomixis	Burgess et al. (2014)
	Crataegus suksdorfii	$2n = 2x$, $3\times$ and $4 \times 2x$ self-incompatible diploid sexual detailss $3\times$ apomicts $4\times$ self-compatible tetraploid pseudogamous apomicts	–	Lo et al. (2009)
	Malus hupehensis	Triploid ($2n = 3x = 51$)	Facultative apomixis	Liu et al. (2014)
	Potentilla indica	Tetraploid (-)	Apomixis	DOBEŠ. et al. (2015)
Melastomeae	*Tibouchina hatschbachii*	- $n = 9$	Apomixis	Maia et al. (2016)
Ranunculaceae	*Anemopaegma acutifolium, A. arvense* and *A. glaucum*	Tetraploid ($2n = 4x = 80$)	Facultative apomixis	(Sampaio. et al. 2013)
	Ranunculus auricomus	2–$4x$	Apomixis	Nogler (1984)
Dryopteridaceae	*Crepis barbigera*	Tetraploid ($2n = 4x = 96$)	Apomixis	Hersh et al. (2016)
Chenopodiaceae	*Beta vulgaris*	Tetraploid ($2n = 3x = 27$)	Facultative apomixis	Szkutnik (2010; Dedong et al. (1999)
Brassicaceae	*Arabis holboellii* (*Boechera holboellii*)	Triploid ($2n = 3x = 21$)	Facultative apomixis	Naumova et al. (2001); Aliyu et al. (2010)
Aspleniaceae	*Asplenium monanthes*	Triploid ($n = 2n = 108$ and $2n = 144$)	Apomixis	Dyer et al. (2012)
Guttiferae	*Hypericum perforatum*	Tetraploid $2n = 4x = 32$	Facultative apomixis	Barcaccia et al. (2006)
Juglandaceae	*Carya cathayensis*	Diploid $2n = 2x = 32$	Apomixis	Zhang et al. (2012)
Myrtaceae	*Psidium cattleyanum*	Polyploid, $n = 11$, $2n = 44$, $2n = 55$, $2n = 66$, $2n = 77$, $2n = 82$ and $2n = 88$	Facultative apomixis	Souza-Pérez and Speroni (2017)
Euphorbiaceae	*Manihot esculenta*	$2n = 38$	Facultative apomixis	Nassar and Collevati (2008)
Cucurbitaceae	*Bryonia alba*	–	Apomixis	Novak and RN Mack (1995)

Table 1 (continued)

Family	Species	Ploidy	Type of apomixis	References
Ulvaceae	*Ulva prolifera*	–	Apomixis	Ogawa et al. (2015)
Gigartinaceae	*Mastocarpus stellatus*	–	Apomixis	Li et al. (2016)
Pteridaceae	*Cheilanthes yavapensis*	Tetraploid ($2n = 4x = 120$)	Apomixis	Grusz et al. (2009); Windham (1993)

involved in apomictic processes in *Boechera holboellii* (Naumova et al. 2001; Sharbel et al. 2009). Moreover, *Boechera holboellii* genome-wide data has been published, and its research on apomictic-related genes has made some progress, laying a foundation for the study of apomixis (Kliver et al. 2018).

Molecular study of apomixis

There are relatively few studies of the molecular regulation of apomixis but some achievements have been made, and these lay a solid foundation for further studies (Table 2). DNA methylation is often associated with apomeiosis, and methylation is always present during subsequent reproduction. The DNA methylation pathway plays an important role in the development of gametophytes, and there are differences in apomictic and sexual reproduction. It has been found that the genes *dmt102* and *dmt103* in ovules are expressed mainly in restricted domains in and around germ cells (Fig. 2e) (Garcia-Aguilar et al. 2010). This suggests DNA methylation pathways are critical for maize gametophyte development during reproduction and may play key roles in apomictic and sexual reproduction. To study the effects of DNA demethylation on reproduction patterns in *Paspalum simplex*, the progeny of apomictic plants treated with the demethylation agent 5′-azacytidine have been analyzed (Fig. 2f). The results indicate the apomictic genus Parthenogenetic inhibitory factor may be inactivated by DNA methylation (Podio et al. 2014). Moreover, modification of DNA methylation is one mechanism through which imprinting may be altered to allow endosperm development in apomicts (Fig. 2l) (Spielman et al. 2003).

Polycomb-group (PcG) has been shown in many studies to be one of the regulatory cores in apomixis. Polycomb-group (PcG) mechanisms are based on two principal types of multiprotein complex, polycomb repressive complex 1 (PRC1) and polycomb repressive complex 2 (PRC2) (Schwartz and Pirrotta 2008). The PRC2 complex is concentrated in Drosophila, mammals and flowering plants. In Drosophila, the PRC2 complex consists of three PcG proteins - an additional sex comb (Esc), an inhibitor of Zeste 12 (Su(Z)12) and an enhancer of Zeste [E(z)] and additional core proteins such as p55. Esc and p55 are WD-40 proteins; Su(z)12 is a C2H2

zinc finger; E(z) is a SET-domain protein that uses methylating histone H3 lysine 27 (H3K27) to silence target gene expression (Schwartz and Pirrotta 2008). The disruption of PRC2 gene expression may result in abnormal seed development in *Arabidopsis* (Hehenberger et al. 2012) and rice (Folsom et al. 2014). *Fertilization-independent seed (FIS)* and *fertilization-independent endosperm (FIE)* are important members of the polycomb group. *FIS* genes play important regulatory roles in the growth and development of plants, especially in the formation of seeds in sexual reproduction. The expression level of this gene is significantly different between sexual and apomictic apple species. From this, it can be inferred that this gene may be involved in the regulation of apomictic apple species (Liu et al. 2012b). In the mutant of *Arabidopsis thaliana* FIS gene, the degeneration of the embryo and the excessive development of endosperm can be observed (Fig. 2i) (Kiyosu. et al. 1999). In tomato, *FIE* silencing results in the formation of abnormal phenotypes during reproductive development. Examples include, increased numbers of sepals and petals, a fused ovule and pistil and parthenocarpic fruit formation (Liu et al. 2012a). In addition, the genes of the polycomb family have been shown to autonomous development of the endosperm in the absence of fertilization is repressed (Fig. 2i) (Kiyosu et al. 1999). AGL62 binds to FIS to form a complex and mediates the 65 cellulogenesis of the endosperm (Fig. 2j) (Hehenberger et al. 2012).

AGO 9 can induce the ovule to produce multiple archespores, this provides a primitive source of cells for the formation of multiple embryos (Fig. 2h) (Olmedo–Monfil et al. 2010). Apomeiosis occurs in the mutant *Arabidopsis thaliana* gene *DYAD/ SWITCH1* (*SWI1*), indicating that the *DYAD/ SWITCH1* gene can regulate meiosis (Fig. 2d). Most fertile ovules in *DYAD* plants form triploid seeds, which are produced by fertilization of unreduced female gamete by a haploid male gamete, which in some cases lead to infertility in offspring. However, the mutation of a single gene leads to functional apomeiosis of the sexual reproductive species, providing a reference for studies leading to apomictic mechanisms (Ravi et al. 2008). In addition, *ORC* is a multiprotein complex which controls DNA replication and cell differentiation during apomixis (Fig. 2g) (Siena et al. 2016). Furthermore, *GID1* may participate in the differentiation of a single megaspore mother cell in ovule development and

Table 2 Apomixis candidate genes and related information

Genes	Description	Affected process? or regulatory aspect?	Species	Reference
Argonaute 9 (AGO 9)	AGO9 controls female gamete formation by limiting the specification of gametophyte precursors in a non-cell-autonomous manner	Diplospory	Arabidopsis, AGO9 mutant (diplospory)	Olmedo-Monfil et al. (2010)
AGO 104	AGO 104 and AGO9 are homologous genes, and their defects can produce a phenotype similar to apomixis, giving rise to up to 70% of functional unreduced female gametes	Diplospory	Tripsacum (diplospory)	Singh et al. (2011)
DYAD/SWITCH1	Result in defects in female meiosis/affect meiosis in megasporocytes	Diplospory	Arabidopsis, dyad mutant (amphimixis)	Agashe et al. (2002); Boateng et al. (2008)
dmt	The expression of the dmt (DNA methyl transferases) gene was significantly different between sexual and apomictic reproduction. The absence of dmt102 and dmt103 can produce a phenotype similar to apomixis	May be involved in differentiation between apomictic and sexual reproduction	maize-Tripsacum hybrid (diplospory)	Garcia-Aguilar et al. (2010)
ORC	ORC (ORIGIN RECOGNITION COMPLEX) is a multiprotein complex which controls DNA replication and cell differentiation in eukaryotes	Adventitious embryony	Paspalum simplex (apospory)	Siena et al. (2016)
GID1	Gibberellin-insensitive DWARF1	Apospory	Brachiaria brizantha (apospory)	Ferreira et al. (2018)
FIE	Fertilization independent endosperm	Autonomous endosperm	Malus hupehensis (facultative apomictic reproduction) /Solanum lycopersicum (amphimixis)	Liu et al. (2012a, 2012b,)
AGAMOUS-LIKE 62	Agamous-like 62 (AGL62) is a member of the MADS-box gene, an increase in its expression level can inhibit the development of the germ and inhibit endosperm cellularization	Inhibit endosperm cellularization	Arabidopsis, fis2 mutant (amphimixis)	Hehenberger et al. (2012)
SERK	Somatic embryogenesis receptor-like kinase	Adventitious embryony	Poa pratensis (amphimixis or apomixis) / Paspalum notatum (obligate apomixis)	Albertini et al. (2005); Podio et al. (2014)
MSP1	Multiple sporocyte controls early sporogenic development	Adventitious embryony	Oryza sativa (amphimixis)	Nonomura (2003)
ASGR–BBML	Multiple copies of the PsASGR-BABY BOOM-like (PsASGR-BBML) gene reside within the apospory-specific genomic region	Apospory	Pennisetum squamulatum (apospory)	Akiyama et al. (2011); Conner et al. (2015)

Table 2 (continued)

| Genes	Description	Affected process? or regulatory aspect?	Species	Reference
lorelei-like/n20gap-1	Encodes a GPI-anchored protein previously associated with apomixis.	Might play a role in the final stages of the apomixis developmental cascade.	*Paspalum notatum* (obligate apomixis)	Felitti et al. (2011)
PnTgs1-like	*PnTgs1-like* (formerly N69) encodes a trimethylguanosine synthase-like protein whose function in mammals and yeast is critical for development, including reproduction	Apospory	*Paspalum notatum* (obligate apomixis)	Siena et al. (2014)
Deficiens	Deficiens is a member of the B class of floral homeotic regulators that have been shown to specify petal and stamen identity according to the ABC model of floral development	Apospory	*Hieracium piloselloides* (apospory)	Guerin et al. (2000)
Apostart	Speculated to participate in the formation of 2n eggs in apomixis	Diplospory	*Medicago sativa* (diplospory)	Barcaccia et al. (2001); Albertini et al. (2005)
RWP	*RWP* is the key gene controlling polyembryony	Adventitious embryony	*Citrus reticulate* (sporophyte apomixis)	Wang et al. (2017)
LOSS OF APOMEIOSIS (LOA)	Responsible for apospory	Apospory	*Hieracium Praealtum* (apospory)	Kotani et al. (2014)
DMC1	Participation in meiosis	Diplospory	*Arabidopsis* (amphimixis)	Zhao et al. (2014)

provide valuable information on the role of *GID1* in sexual and apomictic reproduction (Fig. 2b) (Ferreira et al. 2018). In the nucellar cells of apomictic genotypes, the *Poa pratensis SERK* gene plays a key role in embryo sac development and it may also transfer signaling gene products to compartments other than their typical ones (Albertini et al. 2005). The *Paspalum notatum SERK2* gene is expressed in the nucellar cells of the apomictic genotype during meiosis but, in the sexual genotype, it is expressed only in the megaspore mother cell (Fig. 2k) (Podio. et al. 2014). The *MSP1* gene encodes Leu-rich repeat receptors, such as protein kinase. The function of this gene in rice is to suppress sporogenesis in the surrounding cells and to inhibit anther wall formation. This could be indicated by the *MSP1* expression pattern and the phenotype of loss-of-function mutants. In contrast, the mutation of *MSP1* could give rise to an enormous number of male and female sporocytes, lead to disorders of the anther wall layer formation and result in the disappearance of the tapetum layer (Fig. 2m) (Nonomura 2003).

In addition to the above genes, there are many other genes involved in apomixis. SuperSAGE was used to identify micro-dissected ovules differentially expressing mRNA tags between two diploids (*Boechera stricta* and *B. holboellii*) and two diploid apomicts (*Boechera divaricarpa*). As a result, more than 4000 differentially expressed mRNA tags were screened. During the different developmental stages, 543 alleles were further expressed between sexual and apomeiotic ovules and 69 *holboellii* specific alleles were significantly upregulated in the apomeiotic ovule. The above evidences support the hypothesis of deregulation of the sexual pathway (Sharbel et al. 2009). Molecular characterization of N69 cDNA indicates that it is homologous to trimethylguanosine synthase/PRIP-interacting proteins in yeasts and mammals. These proteins can act as ERK2-controlled transcriptional coactivators, sn(o)RNA mediators and telomerase RNA cap trimethylation. They can also play important developmental roles in mammals and yeasts. Consequently, the N69-extended sequence has been renamed *PnTgs1-like*. Transcription of *PnTgs1-like* in reproductive development is more abundant in floral organs of sexual genotypes than in apomicts. Such a difference is not found in nutritional tissues. Transcription levels are negatively correlated between reproductive tissues of several genotypes and facultative apomixes (Siena et al. 2014). Felitti et al reported that *n20gap-1*, a *lorelei*-like gene, encodes a GPI-anchored protein previously related to apomixis in *Paspalum notatum* (Felitti et al. 2011).

Moreover, small RNA also plays a regulatory role in this process. ARGONAUTE 9-dependent sRNA silencing plays a key role in cell fate in Arabidopsis ovules, whereas epigenetic reprogramming in associated cells is essential for sRNA-dependent silencing in plant gametes (Olmedo-Monfil et al. 2010). In the process of sexual reproduction, hormones are also important control factors involved in the differentiation of fertilized eggs and in mitosis. Some genes related to cytokinin, auxin and brassinoin are significantly upregulated during aposporous apomixis (Galla et al. 2017).
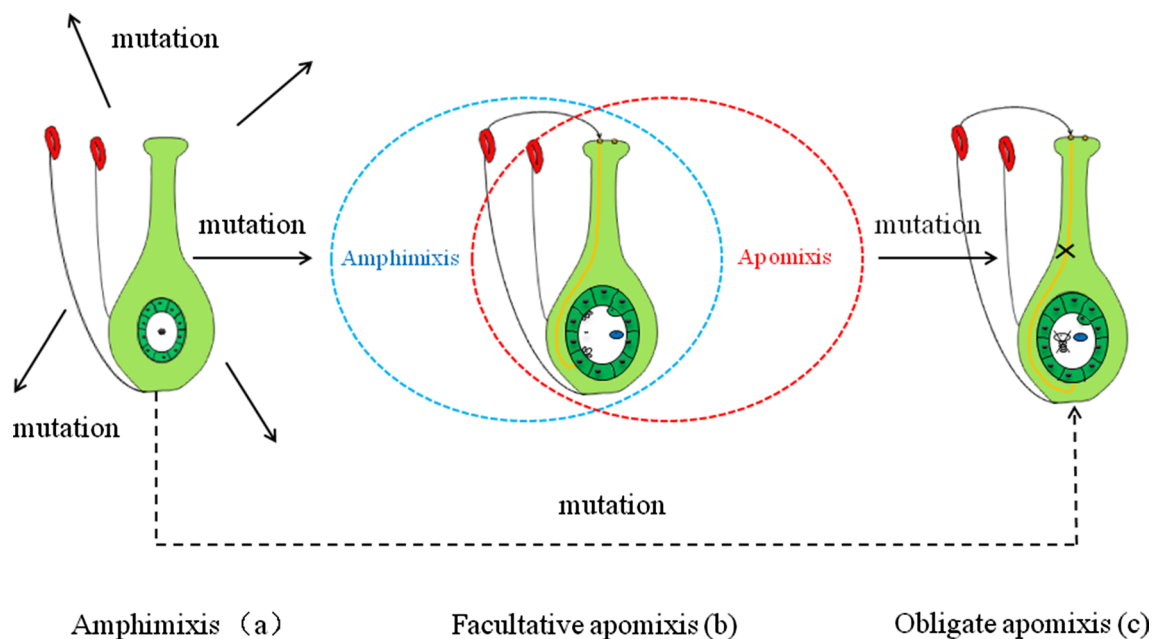
Fig. 3 The emergence of apomictic types. **a** Amphimixis (**b**) Facultative apomixis and (**c**) Obligate apomixis

This shows plant hormones play important roles in apomictic reproduction. At the same time, the role of plant hormones in promoting the differentiation of adventitious buds has been elucidated through tissue culture techniques. Under the stimulation of exogenous steroid hormones (estrone, androsterone, progesterone and epibrassinolide), *Arabidopsis thaliana* can complete the development of endosperm without fertilization (Rojek et al. 2015). So it can be inferred that hormones also play important roles in the apomixis.

Speculation on the emergence of apomixis

Under the well-known double fertilization model, a number of new cases have emerged through mutation and natural selection. Many types of mutation result in plants being unable to produce fertile offspring or being unable to adapt to the environment and thus being eliminated (Fig. 3a). Some of these plant mutations produce apomixis, the macrospore mother cell degenerates and is induced to form an aposporous initial cell or adventitious embryo (Fig. 3b). The former forms an eight-cell embryo through mitosis and the latter develops into an indefinite embryo. Plants exhibit apomixis while retaining sexual reproduction, which is facultative apomixis (Fig. 3b). Obligate apomixis mutations may result in loss of sexual reproduction and obligate apomixis (Fig. 3c). Sexual reproduction direct mutations that produce obligate apomixis may require large numbers of simultaneous gene mutations, and this probability is extremely low (Fig. 3).

Meiosis is a complex process controlled by many factors. The completion of gene delivery is also an important physiological process of gametogenesis. Abnormalities in any meiotic step caused by environmental or genetic factors may affect gamete fertility. For more serious abnormalities, complete infertility can be expected (Boldrini et al. 2006).

In the past, apomixis and sexual reproduction were seen as two separate processes (Ernst 1918). It has subsequently been shown that mutagenesis can induce sexual plants to produce results similar to apomictic traits. This further supports the hypothesis that apomictic and sexual growth may share common elements (Fig. 3b) (Tucker 2003). Disruption of the developmental pathways of seed formation is hypothesized as the cause of apomixis in plants. Both hybridization and polyploidy are mechanisms that cause disruption and are typical of asexual plants (Sharbel et al. 2009). In some respects, it can be inferred that apomixis is a process that bypasses sexual reproduction to form seeds. Apomixis is also realized on the basis of part of the original sexual reproduction. Therefore, apomixis is not an isolated process. To understand its mechanism clearly it is necessary to study it in combination with sexual reproduction.

Although evolution from sexual reproduction to apomixis is relatively rare in eukaryotes, it seems to be an important evolutionary process in plants (Asker and Jerling 1992). Facultative apomixis with both amphimictic and apomictic systems, in the absence of fertilization, can produce offspring. In addition, amphimixis can occur in appropriate environments so ensuring richness in gene communication and thus improving the adaptability of offspring (Fig. 3c). Therefore, facultative apomixis can help plants adapt to complex environments and hence increase the survival rate of the offspring.

Discussion

Steps towards engineering of apomixis

Based on what we known about apomixis, we infer that implementation of apomixis must involve the following:

1. Inhibition of meiosis in the megaspore mother cells. This is needed to retain their entire genetic characteristics and to regulate their direct development into embryos. Inhibition would allow study of the mitotic pathway of megaspore mother cell development without fertilization and enable identification of the key regulatory factors of meiosis in the megaspore mother cell population. The relevant genes and enzymes are activated to induce the formation of embryos from the megaspore mother cells.

2. Induction of mitosis and the differentiation of nucellar cells, which develop directly into embryos. Nucellar cells are an important source of adventitious embryos. Induction of adventitious embryos has become an important element of apomixis technology. Multiple embryos can be developed by induction of embryo formation by nucellar cells. The number of adventitious embryos must be controlled as the formation of too many embryos can lead to weak seedlings, which do not develop into healthy plants. Therefore, genes controlling these qualitative and quantitative traits are required to ensure the effective implementation of apomictic technology.

3. Macrospore mother cells are induced to develop into diplospores by regulating the appropriate genes, so as to bypass pollination and to initiate the direct development of embryos having the same genetic makeup as the mother.

From the data collected so far, we find many species show some apomictic characteristics. While these offer a wealth of material for research, very little effort has yet been focused on elucidating the mechanism of apomixis. The overall control network has yet to be established and only this can elucidate the machinery of apomixis from the functional research of some candidate genes. Unequivocal confirmation of apomixis and the elucidation of its mechanism requires the simultaneous examination of both genetic and cytological evidence. Cytological observation should be used first to identify the

key period(s) of embryonic development and then multi-omics technology could be used to identify a set of candidate genes. Next, model plant functional verification could be used on the gene candidates to understand apomictic gene function, the candidate genes being verified, one by one, to unravel the regulation network of the apomictic mechanism.

Prediction

Apomixis usually occurs in wild species and the ultimate goal of genetic research in apomixis has been to transfer apomixes to their crop relatives (Khan et al. 2015). So far, hundreds of apomictic plants have been found, these provide abundant material for the study of related mechanisms. The Gramineae is one of the most economically important families and contains a large number of key crops. Although apomixis is not found in any of the major graminaceous arable plants there are nevertheless large numbers of apomictic species in this family. These species offer good experimental material for use in the realization of apomixis in cereal crop plants. It is predicted that gramineous crops such as wheat and rice may become the first to achieve apomixis. In the gramineae, many species with apomixis have been found. These include: *Cenchrus ciliaris, Paspalum minus, Poa pratensis, Paspalum Anachyris, Pennisetum glaucum, Brachiaria decumbens, Brachiaria humidicola, Potentilla puberula, Eragrostis curvula, Panicum maximum*. Especially notable is *Poa pratensis*, which belongs to *Poa* subfamily and is closely related to barley and wheat of the same subfamily. It is also characterized by facultative apomixis and so is good material for studying this biological property.

A main component of any study of apomixis must be to better understand the differences between amphimictic and apomictic species from both physiological and also molecular perspectives. Species characterized by facultative apomixis are ideal experimental materials. Species exhibiting facultative apomixis can be used as research material for pollination and for non-pollination treatment. The key period of embryo formation can be determined through cytological observation. *Poa pratensis* (Poaceae) has the characteristics of facultative apomixis, so further study of its apomictic mechanism would likely be worthwhile. Then, the ovules will be stripped off and the ovules of the two materials analyzed in multi-omics, so the candidate genes can be approximately identified. Using a model plant for genetic validation, we will eventually confirm the key genes subsequently. The technology will be achieved through gene knockout, gene modification, overexpression and so on. At the same time, metabolomics and small RNA were studied to better understand the mechanism of apomixis. Many factors involved in the regulation of apomixis were obtained (Fig. 4). A, B, C, D, E, and F represent factors that participate in or that affect apomixis. They represent certain genes, active substances, functional proteins, small RNA, and transcription factors. When the influencing factors of apomictic reproduction of *Poa pratensis* are understood, differences and comparisons with related species, such as barley or wheat, can be made to transfer the missing factors and to inhibit the redundant factors, so as to achieve the construction of apomictic reproductive system in barley (Fig. 4c). Using this apomictic model we should be able to speed the development more efficient graminaceous crops and to reduce breeding costs.

Experimental exploration of apomixis

At this stage, however, no major seed crop species are apomictic and attempts to introduce the apomictic trait into crop plants from their apomictic relatives via conventional breeding schemes have been largely unsuccessful (Barcaccia and Albertini 2013). Nevertheless, to promote the application of related technologies, researchers have undertaken many studies on apomixis. Mutations in three genes *SPO11-1, REC8* and *OSD1* is the best combination of meiotic modification into mitosis in Arabidopsis. At the same time, these three combinations of mutations can effectively convert meiosis into mitosis, thereby producing male and female clonal diploid gametes in rice (Mieulet et al. 2016).

Apomixis has been induced in cassava (cultivar SC5) by treating female flower buds with 1, 1.5 or 2% (v/v) dimethyl sulfoxide (DMSO). The results show the 1.5% DMSO treatment was most effective for inducing apomictic seed formation with the highest percentage of fruit set and the formation of true apomictic seeds (Chen et al. 2018). This study indicates there is an effective technology available for inducing apomixis. Attempts to transfer the apomictic trait to crops from wild relative species have so far resulted in partially fertile, cytogenetically unstable and agronomically unsuitable lines (Podio et al. 2014). In the past few
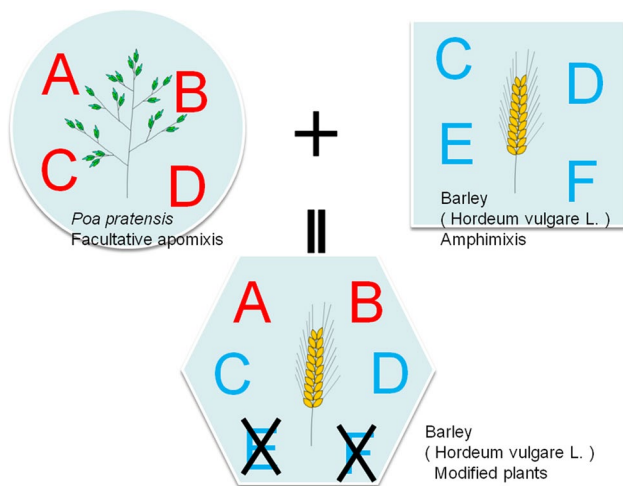
Fig. 4 The realization of apomixis

years, using differential display and RNA-seq approaches, a large number of candidate genes have been identified that are involved in the reproductive organs of sexual plants and their apomictic counterparts. Laspina et al. performed a gene expression differential analysis on the immature inflorescences of Paspalum notatum and apomictic tetraploid genotypes, and finally identified 65 candidate unigenes. A series of sequences related to central cellular processes were subsequently identified by functional classification (Laspina et al. 2008). These efforts provide valuable information for revealing the mechanisms of apomixis. Furthermore, the study of apomixis can also explore endosperm development processes of apomictic species (Ogawa et al. 2013). This will also offer new opportunities for improving the breeding of species in which the endosperm is the main part of the crop. In sexual species, the endosperm typically requires a ratio of two maternal genomes to one paternal genome for normal development but this ratio is often altered in the apomicts. In the course of apomictic reproduction, all the genetic material of the endosperm comes from the mother, hence it is inferred the genotype of the endosperm is fully consistent with that of the mother.

The realization of apomixis technology has greatly promoted the development of agriculture and will bring huge innovations to agriculture, but there are still potential challenges in application. At present, genetic modification of related species has achieved good results, but there are still huge obstacles to genetic modification of distant species. The transformation of distant species is based on an in-depth understanding of the mechanism of apomixis, and the current mechanism of apomixis is still vague, so there is still a long way to go to achieve full application of apomixis.

Conclusions

If apomixis can be engineered into sexual crops in a controlled manner, its future impact on agricultural systems has the potential to be both widespread and profound. Apomixis will allow clonal seed production and this will enable seed quality to be raised and seed production costs lowered. This in turn will lower the input costs of fruit and vegetable production while also raising yields and making them more consistent (Barcaccia and Albertini 2013). It is generally agreed the introduction of apomixis to agronomically important crops will revolutionize agriculture (Pupilli and Barcaccia 2012). A better understanding of the inheritance patterns for apomixis is fundamental for facilitating the identification of candidate genes, which in turn is essential for engineering apomixis into sexual crops (Pupilli and Barcaccia 2012).

In general, apomixis is not conducive to fitness in evolution as it does not foster the propagation of new genes in a population and as it generally serves only to reduce genetic diversity. Many apomictic species retain some ability to reproduce sexually. Here, their apomictic trait enables them to thrive in environments where pollination is impossible but then, when conditions allow, they are also able to resume sexual reproduction. Hence, apomixis offers a species an enhanced ability to adapt to and survive in difficult environments. This characteristic can thus be viewed as a 'refuge' behavior which is a potentially advantageous evolutionary adaptation. In addition, it was found in the data collected that most apomictic plants are polyploid (Table 1). It may provide more opportunities for variation in plants. At the same time, multiple genetic material can attenuate adverse mutations, resulting in a higher proportion of apomixis.

At the core of the study of apomixis is to gain an understanding of the mechanism of embryo formation. The discovery of genes that are specifically or differentially expressed during embryogenesis and embryo sac formation has become key to the study of apomixis. Mastering this will greatly facilitate the realization of apomixis. Based on current research, technical level and the concerted efforts of many researchers, the establishment of apomictic technology systems is expected to be realized this century.

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