Apomixis: Current Status and Future Prospects

Shailendra Goel and Imran Siddiqi

Department of Botany, University of Delhi, Delhi 110007 India. Email: shailendragoel@gmail.com; and Centre for Cellular and Molecular Biology (CCMB), Uppal Road, Hyderabad-500 007 Andhra Pradesh, India. Email: imran@ccmb.res.in

Abstract

Apomixis is a mode of asexual reproduction wherein the maternal genotype is preserved by avoiding recombination and syngamy. Apomixis is common among angiosperms being represented in ~400 genera encompassing ~40 plant families. Potential benefits of apomixis have been explained in various ways, most prominent being fixation of heterosis, maintenance of superior genotypes, and maintaining germplasm without mixing. The potential economic impact of this important trait can be imagined from an estimate of the benefit that would ensue from realizing apomictic technology in rice which was calculated to be US $2.5 billion per annum. This emphasizes the role apomixis can play in development of designer crops. Barring a few exceptions of some forage crops and some instances of adventitious embryony in fruit yielding trees, apomixis does not occur in crop species. Worldwide, most genomic resources for plants have been generated in crop species and together with the complexities inherent in the control of apomixis, this renders the task of translating the present scenario of research in apomixis to actual apomictic crops, a goal that is still somewhat distant. Nevertheless considerable progress has been made in deciphering the mechanisms underlying apomixes. In the last two decades, more apomictic genera are being reported as new methods of detecting apomixis are employed. This is also garnering support for the notion that multiple mechanisms for expression of apomixis do exist, which underlines the complexities in achieving the goal of exploiting apomixis in cultivated crops. This review is aimed at highlighting recent advances in research on gametophytic apomixis with emphasis on Poaceae.

Keywords: Apomixis, asexual reproduction, apospory, diplospory, multigenic trait, epigenetic control

Introduction

Apomixis is classified as sporophytic and gametophytic. Multiple reviews have explained the basis of this classification (Ozias-Akins and Dijk 2007; Bicknell and Koltunow 2004; Koltunow and Grossniklaus 2003; Spillane et al 2001; Grimanelli et al 2001; Crane 2001). Apomixis is common among angiosperms being represented in ~400 genera encompassing ~40 plant families (Carman 1997). Agamospermy is much more common than conceived (Plitman 2002). The economic benefit of this important trait was estimated at US $2.5 billion per annum (McMeniman and Lubulwa 1997). In general, sporophytic apomixis is marked by embryos arising from sporophytic cells through adventitious embryony without the involvement of a gametophyte and exists along with the sexual pathway, while gemetophytic apomixis is the result of alteration in sexual pathway itself and involves the formation of a gametophyte. Gametophytic apomixis is further sub-classified as apospory and diplospory. In apospory, one of the sporophytic cells develops into an unreduced embryo sac and is marked by the presence of multiple embryo sacs. Another feature emphasized about apospory is that the unreduced embryo-sac occupies a nearby position to the megaspore mother cell (MMC) (Koltunow and Grossniklaus 2003) while in diplospory it occupies the same position as MMC since the unreduced embryo sac develops through bypassing meiosis.

Although many taxa and families have been shown to display apomixis, three families, Asteraceae, Rosaceae, and Poaceae have greater representation of apomixis. Most of the studies in last three decades have been performed on Asteraceae and Poaceae largely due to the ease of phenotyping apomictic plants but also due to economic importance of Poaceae. Furthermore, Asteraceae is important in showing the presence of autonomous endosperm while Poaceae requires pseudo-fertilization for the development of endosperm.

Components of apomixis

Successful apomixis requires the following components: avoidance of recombination to maintain maternal genotype, unreduced egg formation by eliminating reduction division, avoidance of fertilization of

unreduced egg, leading to formation of embryo through parthenogenesis and autonomous or pseudogamous endosperm. Studies on apomixis have focused on these four aspects using mainly two approaches to understand the mechanism of apomixis at cellular, cytogenetic and molecular levels - analysis of naturally existing apomicts; and synthesis of apomixis through mutagenesis.

Initial studies in apomixis revolved around cytological investigation which are still employed to understand the apomictic process in various taxa along with newer, more sophisticated tools like flow cytometric analysis of seeds (Matzk et al 2000). Cytological techniques are helpful in understanding megasporogenesis and ovule structure and involve microscopy of cleared ovules (Young et al 1979), a method widely used for phenotyping prospective apomicts. Other approaches to study apomixis include genetic analysis (involving RFLP, AFLP, RAPD, retrotransposons and microsatellites), differential gene expression, deletion studies and more recently, epigenetic studies (Ozias-Akins and Dijk 2007; Pupilli and Barcaccia 2011; Tucker et al 2012; Olmedo-Monfil et al 2012)

Studies on naturally existing apomicts

1. Apomicts in Poaceae

Various Poaceae members have been studied for presence of apomixis over the years which include both apospory (*Brachiaria*, *Panicum*, *Paspalum*, *Pennisetum*, *Cenchrus* and *Poa*) and diplospory (*Tripsacum*). Among these genera, all of which have a pseudogamous endosperm, *Cenchrus*, *Brachiaria*, *Panicum* and *Poa* are forage crops while *Pennisetum* is used as forage and grain crop. *Tripsacum* is a related genus of maize which is a major grain crop. Genetic studies have been carried out in these species to identify markers linked to the locus governing apomixis. Some of the pronounced features in these studies are suppression of recombination, accumulation of transposable elements and hemizygous nature of aposporous locus. These features have contributed to the elusive nature of the genetic basis of apomixis by hampering the progress of map-based cloning efforts.

Suppression of recombination and hemizygosity at aposporous locus

Suppression of recombination around the apomictic locus has been compared to evolution of Y chromosomes wherein features like repression of recombination and accumulation of transposable elements (TEs) are similar to many reported apospory loci (Pupilli et al 2011).

Suppression of recombination has been reported in *Pennisetum*, *Cenchrus* and *Paspalum*. In *Panicum*, although there is no report of recombination suppression, clustering of markers suggests a region of low recombination (Ebina et al 2005; Ozias-Akins and Dijk 2007). *Poa pratensis* is the only genus in Poaceae where recombination has been reported between loci governing two key components, apospory and parthenogenesis (Albertini et al 2001). Lack of recombination has been often linked to chromosomal rearrangements or heterochromatization of the loci and hemizygous nature of the apomixis locus. Although there are examples of each of these (and in some case more than one can be attributed to the apomixis locus) no consensus has been identified. For example, an inversion was identified as the reason of suppression of recombination in *Pennisetum squamulatum*, while in *Cenchrus ciliaris* the reason was pericentric location of the loci (Goel et al 2003). The aposporic locus in these two species is located in a heterochromatic region but in *Paspalum simplex*, the apomixis locus is situated in heterochromatic-poor region (Calderini et al 2006). The location of apospory locus in different *Pennisetum* and *Cenchrus* species was compared and the location of apospory locus was found to vary in different species. Differences in the amount of repeat sequences and hetrochromatic nature of apospory locus carrying chromosome were also noticed (Akiyama et al 2011). This also suggests that the apospory locus might be changing its location. Presence of polyploidy along with apomixis provides tolerance to these chromosomal changes. Similar chromosomal rearrangements have also been observed in *Paspalum* (Pupilli et al 2011). It is also suggested that large-scale chromosomal rearrangements and small-scale arrangements may have played a role in establishment of apomixis after its origin. Apomixis inhibits recombination, especially around the locus governing the trait itself, leading to accumulation of repeat elements and transposons causing the heterochromatization of locus and imparts a hemizygous nature to it.

Synteny of aposporous locus among apomictic species and with Poaceae

Many attempts have been made to identify syntenic regions of apomictic locus by using information available in rice and maize. Lack of DNA sequences in apomictic grasses has been a limiting factor due to which, earlier attempts of comparative mapping were carried out using RFLP (Grimanelli et al 1998; Pessino et al 1997; Pupilli et al 2001). The aposporous locus in *Brachiaria brizantha* has been shown to be syntenic with chromosome 2 of rice and chromosome 5 of maize (Pessino et al 1997, Ozias-Akins and Dijk 2007). Investigations in three apomictic species of *Paspalum* (*P. simplex*, *P. malacophyllum* and *P. notatum*) showed retention of similar apomixis linked markers. In *P. simplex* and *P. malacophyllum*, apomixis controlling

locus (ACL) was syntenic with a part of rice chromosome 12 while in *P. notatum*, the region proximal to ACL maintained synteny with rice chromosome 12 but the distal region was syntenic to chromosome 2 indicating a heterozygous translocation (Pupilli et al 2004). Use of common molecular probes in different apomictic species has emphasized lack of common genomic region (Grimanelli et al 2001) between different apomictic species suggesting an independent origin of apomixis.

Availability of some BAC sequences in *Pennisetum* allowed exploration of synteny of apospory locus with rice leading to identification of synteny with chromosome 11 of rice, but when more sequences were generated from the apomixis specific genomic region (ASGR), the synteny did not hold and only microsyntenic regions could be identified (Gualtieri et al 2006, Conner et al 2008). A similar condition was observed when syteny was explored between ASGR and *Sorghum bicolor* (Conner et al 2008). Sequence analysis by Conner et al (2008), also revealed that only 23-40% of sequences are repetitive elements which was surprising considering earlier reports of abundance of some repetitive elements at ASGR (Akiyama et al 2004). The ASGR seems to harbour multiple small regions showing synteny with multiple regions from rice and sorghum (Conner et al 2008). A later study showed that the opie-2 retrotransposon sequences which were once believed to be closely associated with ASGR (Akiyama et al 2004) are not closely associated with it when investigated in other related apomictic *Pennisetum* species (Akiyama et al 2011).

Expression studies

Besides genetic studies, expression profiling has also been performed to understand the differences between sexual and apomictic plants. The most commonly used method employed for the purpose is differential display followed by cDNA-AFLP and RT-PCR. These methods are prone to innate heterozygosity existing in apomicts. This drawback has prompted confirmation of the variation identified between sexual and apomictic floret, which was achieved through analysis of expression by a method not affected by sequence polymorphism (e.g. Northern and microarray) or mapping of identified fragments to analyse their linkage with apomictic locus.

In *Brachiaria*, differential display was used to identify two fragments specific to apomictic cDNA (Leblanc et al 1997). In another study on the same plant, different stages of sporogenesis and gametogenesis from sexual and apomictic plants were used for differential display leading to identification of 11 differentially expressed fragments (Rodrigues et al 2003). In both studies, none of the fragments were linked with the apomixis locus.

Differential display in *Paspalum* identified three fragments which did not show any linkage to the apomictic trait (Pessino et al 2001). Another study in *P. notatum* (Laspina et al 2008) identified 65 differentially expressed genes between sexual and apomicts. Their analysis indicated involvement of a signal transduction cascade but did not identify any transcript showing direct functional relationship with apospory (Laspina et al 2008). cDNA-AFLP was used to identify apomixis linked alleles (Polegri et al 2010) in *P. simplex*. These alleles had nonsense and frameshift mutations suggesting a pseudogene nature for them. These alleles might exist because natural selection has little chance to remove them owing to a combination of lack of recombination and apomixis (Pupilli and Barcaccia, 2011).

cDNA AFLP was also used in *Poa pratensis* to identify 179 transcript-derived fragments showing differences between sexual and apomict ovaries, but none was identified with an exclusive expression and showed differences in only timing of expression (Albertini et al 2004, Albertini et al 2005).

In *Panicum maximum*, expression patterns between sexual and apomictic ovules identified a transcript showing variation in expression between ovules of sexual and apomictic plants (Chen et al 2005). Another study used microarray analysis and RT PCR to identify 2 EST showing differential expression between apomict and sexual ovaries but no attempts were made to assign them to apomixis locus (Yamada-Akiyama et al 2009).

Differential display in *Cenchrus ciliare* (*Pennisetum cilare*) identified two genes but none was linked to the apospory locus (Singh et al 2007). Zhen et al (2011) has used next generation sequencing (NGS) in an attempt to identify candidate genes responsible for the apomixis trait in *Pennisetum*. They sequenced transcripts from apomictic ovaries and tried to map it to the ASGR carrier chromosome. They identified 48 transcripts which were ASGR carrier chromosome-specific but only one was tightly linked with ASGR.

All these studies identified several genes but most of them were either differentially modulated or showed quantitative variation suggesting that these genes act downstream of the switch responsible for conversion to apomictic mode of reproduction. It also support the hypothesis that apomixis results from deregulation of the sexual pathway (Albertini et al 2004, 2005). Nevertheless, functional analysis of genes which map to the apomictic locus can provide invaluable insights to the understanding of apomixis.

2. Apomixis in other families

Besides Poaceae, apomixis has been extensively studied in the family Asteraceae in genera *Erigeron*, *Taraxacum* and *Hieracium*. Most commonly studied species are *Erigeron annuus*, *Taraxacum officinale* and *Hieracium pilosella*. These genera are polyploid diplosporous apomicts with apomictic polyploids and sexual diploids existing in the same species. Other shared features are autonomous endosperm development and suppression of recombination but *Erigeron* and *Taraxacum* showed the presence of unlinked diplosporous and parthenogenesis loci (Noyes et al 2007; Dijk and Bakx-Schotman 2004). In *Hieracium*, a deletion study could identify two loci governing apomeiosis and parthenogenesis (Catanach et al 2006).

Some other commonly used models are *Boechera* (also called *Arabis*) from family Brassicaceae and emerging model *Hypericum perforatum* (Clusiaceae). Among them, *Boechera*, can be of importance because it belongs to the same family as *Arabidopsis thaliana*, a plant with a well characterized genome and a vast pool of mutants which can be valuable for functional analysis of apomictic candidate genes.

Synthesis of apomixis through mutagenesis

Analysis of naturally existing apomixis showed that although most apomictic loci behave as a dominant single locus, the structure of the locus itself is complicated. These analyses supported the view that apomixis might be a multigenic trait and there are different components which together constitute the trait of apomixis. Synthesis of apomixis is possible by creating these different components of apomixis and combining them. Earlier, this approach was not popular but it is one of the most promising approaches after a couple of successes (Ravi et al 2008; dÉrfurth et al 2009; Marimuthu et al 2011). Over time, a number of mutants have been reported which partially mimicked one or the other component of apomixis. These mutants can be classified by following the categories of apomictic components (apomeiosis, parthenogenesis and endosperm) explained earlier.

Mutants mimicking apomeiosis

Apomeiosis refers to failure or avoidance of meiosis and recombination to preserve maternal genotype. The foremost requirement of apomixis is failure of meiosis to produce a diploid egg cell. A number of mutants have been reported which mimic either aposporous phenotype (multiple archesporial cells) or diplosporous phenotype (failure of meiosis). A mutation reported in maize by Sheridan et al (1996), resulted in *m*ultiple *a*rchesporial *c*ells (*MAC1*). In rice, *m*ultiple *sp*orocyte1 (*MSP1*) has a similar function (Nonomura et al 2003).

Among mutations mimicking diplosporous phenotype, probably the well-studied mutant is *switch1/dyad* in *Arabidopsis*. Strong alleles of the *swi1* gene cause replacement of the two divisions of female meiosis by a single equational and a loss of sister chromatid cohesion in the case of male meiosis. A weak allele of *swi1*, called *dyad* affects only female meiosis and leads to formation of some functional apomeiotic female gametophytes (Ravi et al 2008). The maize orthologue of *swi1* is *ameiotic* (*am1*), and am1 mutants also replace meiosis with mitosis (Pawlowski 2009). Olmedo-Monfil et al (2010) reported the *ago9* mutation in *Arabidopsis* which produces a multiple spore phenotype which is similar to apospory. Singh et al (2011) reported another mutation, *Dnr4* in maize which encodes AGO104 protein and results in non-reduced gametes. All these mutation result in production of a few unreduced female gametophytes along with reduced female gametophytes. Recently, success was achieved in production of only unreduced gametes in *Arabidopsis* with *MIME* (*M*itosis *I*nstead of *Me*iosis) genotype (dÉrfurth et al 2009), which is a triple mutant (*Atspo11*, *Atrec8*, *osd1*). The *Atspo11* mutation prevents chromosome pairing and recombination, while *Atrec8* modifies chromatid segregation and osd1 results in omission of the Meiosis II division. The overall effect of the combination of the three mutants is to convert meiosis into mitosis. These mutations affect both male and female meiosis and differ from naturally occurring apomixis in complete elimination of normal gametogenesis which is a part of normal sexual pathway. Another encouraging achievement has been the production of functional apomictic clones if apomeiosis in *MIME* and *dyad* alleles is combined with uniparental genome elimination following fertilization as occurs in *cenh3* mutant lines that express a manipulated CENH3 (TAILSWAP) protein (Marimuthu et al 2011).

Mutants mimicking parthenogenesis

Parthenogenesis normally leads to haploid production (Dunwell 2010). One of the earliest known mutations was *hap* (Hagberg and Hagberg 1980) which was induced in barley through EMS mutagenesis and resulted in low frequency of haploid induction. The Salmon system of wheat comprises three isogenic alloplasmic lines with either zygotic (aS) or autonomous, fertilisation-independent (cS kS) embryo development. In wheat *Salmon* system, (a 1BS/1RS wheat rye translocation) is known to show parthenogenesis. Exact mechanism in this system is not known but 1BS is believed to have a parthenogenesis suppressing gene while 1RS is postulated to have a parthenogenesis inducing gene (Matzk et al 1995). Mutation of MULTICOPY SUPPRSSOR OF IRA 1 (*MSI1*) has been shown to lead to initiation of embryogenesis without fertilization; however the

embryos abort early in development (Guitton and Berger 2005). Another mutation called *ig1* (*indeterminate gametophyte*) in maize affects polarity of female gametophyte and causes increased number of eggs, synergids and polar nuclei (Lin et al 1978).

A number of transcription factors viz. BABY BOOM (BBM) (Boutilier et al 2002), LEAFY COTYLEDON (LEC1) (Lotan et al 1998), LEC2 (Stone et al 2001) and WUSHEL (Zuo et al 2002) are known to promote somatic embryogenesis. None of these express either in egg cell or embryo (Curtis and Grossniklaus 2008) although there are exceptions like *SERK1* (SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1) which expresses in wild type ovules.

Mutants mimicking endosperm development: Endosperm is the source of nutrition for the developing embryo and is also the nutritive tissue for human consumption in the cereal and other crops. Among natural apomicts, plants have developed various ways to ensure normal development of endosperm ranging from modification of embryo sac structure to high tolerance of maternal:paternal genome ratio (Ozias-Akins 2006). Among artificial mutants, the *FIS* class mutants allow partial development of endosperm without fertilization. These mutants include *mea*/*fis1* (*medea/fertilization independent seed*), *fis2* (Choudhary et al 1997; Grossniklaus et al 1998), and *fie* (*fertilization independent endosperm*) (Ohad et al 1996). These genes suppress endosperm formation in the absence of fertilization (Grimanelli et al 2001).

All *FIS* class mutants show defects in embryo and endosperm growth suggesting that *FIS* genes are essential for normal development of embryo and endosperm (Chaudhary et al 2001, Grossniklaus et al 1998). Analysis of *FIS* class genes show that they belong to *polycomb* group (PcG) and may play a role in epigenetic control of chromatin (Koltunow and Grossniklaus 2003). *FIS2* functions downstream to *DEMETER* (*DME*), a key regulator of imprinting, further supporting this notion (Choi et al 2002).

Epigenetic studies

Among naturally occurring apomicts, the aposporous locus has been often found to be located in a heterochromatic region rich in transposons raising the possibility of epigenetic control of apomixis. Slotkin et al (2009) have shown the involvement of 21 nt siRNA in heterochromatic programming of sperm cells. Such a mechanism has not yet been identified in the female gametophyte but two mutations which mimic the aposporous phenotype implicate role of RNAi machinery in female gametophyte development and consequently in apomixis. First of these mutations is *ago9*, which encodes the ARAGONAUTE 9 (AGO9) protein and was reported in *Arabidopsis* (Olmedo-Monfil et al 2010). The *ago9* mutation results in multiple gametic cells, a phenotype which resembles apospory. The second mutation of *ago104* was reported in maize and mimics diplospory by causing failure of meiosis. Both AGO104 and AGO9 proteins accumulate in sporophytic cells in the vicinity of gametic precursor cells suggesting that mobile signals from somatic cells influence development of the gametophyte. A recent report has compared sexual and apomictic lines of *Pennisetum glaucum*. The apomictic line had two additional chromosomes introgressed from *P. squamulatum*, conferring the apomictic trait. This analysis discovered sRNA population unique to apomict lines, although further analysis is required to establish their role in control of apomixis (Singh et al 2012).

Conclusion and future prospects

Considerable success has been achieved in recent years in the area of apomictic research. Both approaches of identifying genes responsible for apomixis from natural apomicts and synthesis of apomixis through mutagenesis have made substantial progress. Expression of reporter genes has shown that sexual and apomictic pathways are interrelated emphasizing the need to understand the basis of sexual pathway. Understanding of genetic factors regulating both sexual and apomictic pathways is required to synthesize this trait in crop plants.

The comparative mapping studies suggest distinct genetic origin of apomixis in different plants cautioning against a generalized approach to study apomixis in different species. The understanding of this trait in different apomictic species might be the key to achieve success in synthesizing this trait in different crop species because genetic factors controlling the trait as complex as apomixis, might not be conserved in distantly related species. More advanced tools like deletion mapping and NGS can help to cross the mapping hurdles and identify the elusive genes controlling apomixis. NGS can also help to overcome the problem of lack of genomic resources in species where apomixis is being studied. It is possible that in many of the species, genomic region regulating apomixis is degenerated and has only a few genes. It is also possible that these genes might be epigenetic regulators since many of the recent reports emphasize the role of smRNA in reproductive pathways. The task of analysing apomixis does not end with identifying apomixis controlling factors but will also require finding ways to assemble them in an economically beneficial ways in the crops of importance.

REFERENCES

Akiyama Y, Conner JA, Goel S, Morishige D and Mullet JE. 2004. High-resolution physical mapping in *Pennisetum squamulatum* reveals extensive chromosomal heteromorphism of the genomic region associated with apomixis. *Plant Physiol* 134**:** 1733–1741.

Akiyama Y, Goel S, Conner JA, Hanna WW, Yamada-Akiyama H and Ozias-Akins P. 2011. Evolution of the apomixis transmitting chromosome in *Pennisetum*. *BMC Evol Biol* 11: 289.

Akiyama Y, Hanna WW and Ozias-Akins P. 2005. 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* 11: 1042–1051.

Albertini E, Marconi G, Barcaccia G, Raggi L and Falcinelli M. 2004 Isolation of candidate genes for apomixis in *Poa pratensis* L. *Plant Mol Biol* 56: 879-894.

Albertini E, Marconi G, Reale L, Barcaccia G, Porceddu A Ferranti F and Falcinelli M. 2005. *SERK* and *APOSTART* Candidate genes for apomixis in *Poa pratensis*. *Plant Physiol* 138: 2185-2199.

Albertini E, Porceddu A, Ferranti F, Reale L and Barcaccia G. 2001. Apospory and parthenogenesis may be uncoupled in *Poa pratensis*: a cytological investigation. *Sex Plant Reprod* 14: 213–217.

Bicknell RA and Koltunow AM. 2004. Understanding apomixis: recent advances and remaining conundrums. *Plant Cell* 16: S228–245.

Boutilier K, Offringa R, Sharma VK, Kieft H, Ouellet T, Zhanga L, Hattori J, Liua C-M, Lammeren AMV, Miki BLA, Custersa JBM and Campagne MML. 2002. Ectopic expression of BABY BOOM triggers a conversion from vegetative to embryonic growth. *Plant Cell* 14: 1737-1749.

Calderini O, Chang SB and Jong H. 2006. Molecular cytogenetics and DNA sequence analysis of an apomixis-linked BAC in *Paspalum simplex* reveal a non pericentromere location and partial microcolinearity with rice. *Theor Appl Genet* 112: 1179-1191.

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

Catanach AS, Erasmuson SK, Podivinsky E, Jordan BR and Bicknell R. 2006. Colloquium paper: Deletion mapping of genetic regions associated with apomixis in *Hieracium*. *Proc Natl Acad Sci USA* 103: 18650–18655.

Chaudhury AM, Koltunow A, Payne T, Luo M, Tucker MR, Dennis ES and Peacock WJ. 2001. Control of early seed development. *Annu Rev Cell Dev Biol* 17: 677–699.

Chaudhury AM, Ming L, Miller C, Craig S, Dennis ES and Peacock WJ. 1997. Fertilization independent seed development in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* 94: 4223-4228.

Chen L, Guan L, Seo M, Hoffmann F and Adachi T. 2005. Developmental expression of ASG- 1 during gametogenesis in apomictic Guineagrass (*Panicum maximum*). *J Plant Physiol* 162: 1141-1148.

Choi Y, Gehring M, Johnson L, Hannon J, Harada JJ et al. 2002. DEMETER, a DNA glycosylase domain protein, is required for endosperm gene imprinting and seed viability in *Arabidopsis*. *Cell* 110: 33–42.

Conner JA, Goel S, Gunawan G, Cordonnier-Pratt MM, Johnson VE, Liang C, Wang H, Pratt LH, E. Mullet J, DeBarry J, Yang L, Bennetzen JL, Klein PE, and Ozias-Akins P. 2008. Sequence analysis of bacterial artificial chromosome clones from the apospory-specific genomic region of *Pennisetum* and *Cenchrus*. *Plant Physiol* 147: 1396–1411.

Crane CF. 2001. *Classification of apomictic mechanisms. DF: CIMMYT, IRD, Eur Comm* DG VI (FAIR), Mexico.

Curtis MD and Grossniklaus U. 2008 Molecular control of autonomous embryo and endosperm development. *Sex Plant Reprod* 21: 79–88.

d'Erfurth I, Jolivet S, Froger N, Catrice O, Novatchkova M, Mercier R. 2009. Turning meiosis into mitosis. *PLoS Biol* 7: e1000124.

Dijk PV and Bakx-Schotman J. 2004. Formation of unreduced megaspores (diplospory) in apomictic dandelions (*Taraxacum officinale*, s.l.) is controlled by a sex-specific dominant locus. *Genetics* 166: 483–492.

Dunwel JM. 2010. Haploids in flowering plants: origins and exploitation. *Plant Biotech J* 8**:** 377–424.

Ebina M, Nakagawa H, Yamamoto T, Araya H, Tsuruta S, Takahara M and Nakajima K. 2005. Co-segregation of AFLP and RAPD markers to apospory in Guineagrass (*Panicum maximum* Jacq.). *Grassl Sci* 51: 71–78.

Goel S, Chen Z, Conner JA, Akiyama Y, Hanna WW and Ozias-Akins P. 2003. Physical evidence that a single hemizygous chromosomal region is sufficient to confer aposporous embryo sac formation in *Pennisetum squamulatum* and *Cenchrus ciliaris*. *Genetics* 163: 1069-1082.

Grimanelli D, Leblanc O, Espinosa E, Perotti E, Leon DGD and Savidan Y. 1998. Mapping

diplosporous apomixis in tetraploid *Tripsacum*: one gene or several genes? *Heredity* 80: 33-39.

Grimanelli D, Leblanc O, Perotti E and Grossniklaus U. 2001. Developmental genetics of gametophytic apomixis. *Trends Genet* 17: 597–604.

Grossniklaus U, Koltunow AM and Lookeren CMV. 1998. A bright future for apomixis. *Trends Plant Sci* 3: 415–416.

Grossniklaus U and Schneitz K. 1998. Genetic and molecular control of ovule development and megagametogenesis Semin. *Cell Dev Biol* 9: 227–238.

Gualtieri G, Conner JA, Morishige D, Moore L, Mullet J and Ozias-Akins P. 2006. A segment of the apospory specific genomic region (ASGR) is highly microsyntenic not only between the apomicts *Pennisetum squamulatum* and *Cenchrus ciliaris*, but also with a rice chromosome 11 cis-centromeric genomic region. *Plant Physiol* 140: 963–971.

Guitton AE and Berger F. 2005. Loss of MULTICOPY SUPPRESSOR OF IRA 1 produces nonviable parthenogenetic embryos in *Arabidopsis*. *Curr. Biol* 15: 750-754.

Hagberg A and Hagberg G. 1980. High frequency of spontaneous haploids in the progeny of an induced mutation in barley. *Hereditas* 93: 341-343.

Koltunow AM and Grossniklaus U. 2003a. Apomixis: A developmental perspective. *Annu Rev Plant Biol* 54: 547–574.

Laspina N, Vega T, Seijo JG, Gonzalez AM, Martelotto LG et al. 2008. Gene expression analysis at the onset of aposporous apomixis in *Paspalum notatum*. *Plant Mol Biol* 67: 615-628.

Leblanc O, Armstead I, Pessino S, Ortiz JPA and Evans C. 1997. Nonradioactive mRNA fingerprinting to visualize gene expression in mature ovaries of *Brachiaria* hybrids derived from *B. brizantha*, an apomictic tropical forage. *Plant Sci* 126: 49–58.

Lin BY. 1978. Structural modifications of the female gametophyte associated with the indeterminate gametophyte (ig) mutant in maize. *Can J Genet Cytol* 20: 249–257.

Lotan T, Ohto M, Yee KM, West MAL, Lo R, Kwong RW**,** Yamagishi K**, Fischer** RL**, Goldberg** RB **and Harada JH.** 1998. *Arabidopsis* LEAFY COTYLEDON1 is sufficient to induce embryo development in vegetative cells. *Cell* 93: 1195–1205.

Ravi R, Marimuthu MPA and Siddiqi I. 2008. Gamete formation without meosis in *Arabidopsis*. *Nature* 451: 1121-1124.

Marimuthu MPA, Jolivet S, Ravi M, Pereira L, Davda JN, Cromer L, Wang L, Nogué F, Chan SWL, Siddiqi I and Mercier R. 2011. Synthetic clonal reproduction through seeds. *Science* 331: 876.

Matzk F, Meister A and Schubert L. 2000. An efficient screen for reproductive pathways using mature seeds of monocots and dicots. *Plant J* 21: 97-108.

Matzk F, Meyer HM, Baumlein H, Balzer HJ and Schubert I. 1995. A novel approach to the analysis of the initiation of embryo development in Gramineae. *Sex Plant Reprod* 8: 266-272.

McMeniman S and Lubulwa G. 1997. Project development assessment: an economic evaluation of the potential benefits of integrating apomixis into hybrid rice. *Canberra Economic Evaluation Unit ACIAR* 28: 1-26.

Nonomura KI, Miyoshi K, Eiguchi M, Suzuki T and Miyao A. 2003. The MSP1 gene is necessary to restrict the number of cells entering into male and female sporogenesis and to initiate anther wall formation in rice. *Plant Cell* 19: 2583-2594.

Noyes RD, Baker R and Mai B. 2007. Mendelian segregation for two-factor apomixis in *Erigeron annuus* (Asteraceae). *Heredity* 98: 92–98.

Ohad N, Margossian L, Hsu Y-C, Williams C, Repetti P et al. 1996. A mutation that allows endosperm development without fertilization. *Proc Natl Acad Sci USA* 93: 5319–5324.

Olmedo-Monfil V. Durán-Figueroa M. Arteaga-Vázquez M, Demesa-Arévalo E, Autran D Grimanelli R. Slotkin K, Martienssen RA and Vielle-Calzada J-P. 2010. Control of female gamete formation by a small RNA pathway in *Arabidopsis*. *Nature* 464: 628–632.

Ozias-Akins P. 2006. Apomixis: Development characteristics and genetics. *Crit Revu Plant Sci* 25: 199-214.

Ozias-Akins P, Roche D and Hanna WW. 1998. Tight clustering and hemizygosity of apomixis-linked molecular markers in *Pennisetum squamulatum* implies genetic control of apospory by a divergent locus which may have no allelic form in sexual genotypes. *Proc Natl Acad Sci USA* 95: 5127–5132.

Ozias-Akins P and Dijk PJV. 2007. Mendelian genetics of apomixis. *Annu Rev Genet* 41: 509–537.

Pawlowski WP, Wang CJR, Golubovskaya IN, Szymaniak JM, Shi L, Hamant O, Zhu T, Harper L, Sheridane WF and Cande WZ. 2009. Maize AMEIOTIC1 is essential for multiple early meiotic processes and likely required for the initiation of meiosis. *Proc Natl Acad Sci USA* 106: 3603–3608.

Pessino SC, Espinoza F, Martinez FJ, Ortiz JPA, Valle EM and Quarín CL. 2001. Isolation of

cDNA clones differentially expressed in flowers of apomictic and sexual *Paspalum notatum*. *Hereditas* 134: 35–42.

Pessino SC, Ortiz JPA, LeblancO, Valle CB, Evans C and Hayward MD. 1997. Identification of a maize linkage group related to apomixis in *Brachiaria*. *Theor Appl Genet* 94: 439-444.

Plitman U. 2002. Agamospermy is much more common than conceived: A hypothesis. *Isr J Plant Sci* 50: 111–117.

Polegri L, Calderini O, Arcioni S and Pupilli F. 2010. Specific expression of apomixis-linked alleles revealed by comparative transcriptomic analysis of sexual and apomictic *Paspalum simplex* Morong flowers. *J .Exp.Bot* 61: 1869-1883.

Pupilli F, Labombarda P, Caceres ME, Quarin CL and Arcioni S. 2001. The chromsome segment related to apomixis in *Paspalum simplex* is homoeologous to the telomeric region of the long arm of rice chromosome 12. *Mol Breed* 8: 53-61.

Pupilli F and Barcaccia G. 2012. Cloning plants by seeds: Inheritance models and candidate genes to increase fundamental knowledge for engineering apomixis in sexual crops. *J Biotechnol* 30;159(4):291-311.

Pupilli F, Martinez EJ, Busti A, Calderini O, Quarin CL and Arcioni S. 2004. Comparative mapping reveals partial conservation of synteny at the apomixis locus in *Paspalum* spp. *Mol Genet Genomics* 270: 539-548.

Rodrigues JCM, Cabral GB, Dusi DMA, Mello LVD, Rigden DJ and Carneiro VT. 2003. Identification of differentially expressed cDNA sequences in ovaries of sexual and apomictic plants of *Brachiaria brizantha*. *Plant Mol Biol* 53: 745–757.

Sheridan WF, Avalkina NA, Shamrov II, Batygina TB and Golubovskaya IN. 1996. The *Mac1* gene: controlling the commitment to the meiotic pathway in maize. *Genetics* 142: 1009–1020.

Singh HD, Joshi G, Bhardwaj AR, Agarwal SK, Agarwal M, Jagannath A, Ozias-Akins P, Hanna WW, and Goel S LVD. 2012. Profiling of smRNAs unique to apomictic addition lines in *Pennisetum glaucum*. *Int Plant Animal Genome Conf* XX, 14-18 January, San Diego, CA, USA

Singh M, Burson BL and Finlayson SA, 2007. Isolation of candidate genes for apomictic development in buffelgrass (*Pennisetum ciliare*). *Plant Mol Biol* 64: 673-682.

Singh M, Goel S, Meeley RB, Dantec C, Parrinello H, Michaud C, Leblanc O and Grimanelli D. 2011. Production of viable gametes without meiosis in maize deficient for an ARGONAUTE protein. *Plant Cell* 23: 443-458

Slotkin RK, Vaughn M, Borges F, Tanurdzić M, Becker JD, Feijó JA and Martienssen RA. 2009. Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. *Cell* 136**:** 461–472.

Spillane C, Stimer A and Grossniklaus U. 2001. Apomixis in agriculture: the quest for clonal seeds. *Sex Plant Reprod.* 14: 179–187.

Stone SL, Kwong LW, Yee KM, Pelletier J, Lepiniec L, Fischer RL, Goldberg RB and Harada JJ. 2001. *LEAFY COTYLEDON2* encodes a B3 domain transcription factor that induces embryo development. *Proc. Natl Acad Sci USA* 98: 11806–11811.

Tucker MR, Okada T, Hu Y, Scholefield A, Taylor JM and Koltunow AMG. 2012. Somatic small RNA pathways promote the mitotic events of megagametogenesis during female reproductive development in *Arabidopsis*. *Development* 139: 1399-1404.

Yamada-Akiyama H, Akiyama Y, Ebina M, Xu QS, Tsuruta SI, Yazaki J, Kishimoto N, Kikuchi S, Takahara M, Takamizo T, Sugita SI and Nakagawa H. 2009. Analysis of expressed sequence tags in apomictic guineagrass (*Panicum maximum*). *J Plant Physiol* 166: 750-761.

Young BA, Sherwood RT and Bashaw EC. 1979. Cleared-pistil and thick sectioning techniques for detecting aposporous apomixis in grasses. *Can J Bot* 57: 1668–1672.

Zeng Y, Conner JA and Ozias-Akins P. 2011 Identification of ovule transcripts from the apospory-specific genomic region (ASGR)-carrier chromosome. *BMC Genomics* 12: 206.

Zuo J, Niu QW, Frugis G and Chua NH. 2002. The WUSCHEL gene promotesvegetative-to embryonic transition in *Arabidopsis*. *Plant J* 30: 349–359.

Citation: Goel S and Siddiqi I. 2013. Apomixis: current status and future prospects. In: Muralidharan K and Siddiq EA, eds. 2013. *International Dialogue on Perception and Prospects of Designer Rice*. Society for Advancement of Rice Research, Directorate of Rice Research, Hyderabad 500030, India, pp 147-154.