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Engineering apomixis in rice

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Apomixis, a natural mode of clonal reproduction through seeds, has a vast potential to revolutionize agriculture by virtue of its capacity for fixation of hybrid vigour, and is regarded as a next-generation breeding strategy[1]. Although widespread in the plant kingdom, natural apomixis is generally absent in agriculturally important crops. Hence, efforts are being made to introduce apomixis in agriculturally important crops through strategies such as introgression and mutation[2].

An 'evaluation and synthesis' approach has been followed to understand and induce apomixis in important crops in recent years[1,3]. The evaluation approach had generated information on genetics and molecular biology of apomixis based on studies conducted in natural apomictic systems (such as *Paspalum*, *Pennisetum*, *Panicum*, *Cenchrus*, *Tripsacum*, *Taraxacum*, *Hypericum*, *Hieraceum*, *Eragrostis*). This knowledge is being utilized for synthesis of the phenomenon in otherwise sexual systems, such as rice and *Arabidopsis*. In contrast to sexual reproduction which follows the double-fertilization process of syngamy between meiotically reduced male gametes with reduced egg cell and polar nuclei to generate viable embryo (2*n*) and endosperm (3*n*), it is now well understood that the process of apomictic reproduction essentially involves three components, viz. apomeiosis (formation of unreduced female gamete), parthenogenesis (development of egg cell without fertilization) and functional endosperm development[4]. Molecular progression of these components generates meiotically unreduced (2*n*) egg cells to develop without fertilization, thereby bypassing meiotic crossing-over and fertilization events – the two stages of creating variability – eventually leading to development of a clonal embryo[1,4]. Furthermore, in contrast to earlier reports suggesting a major locus control of apomixis, the three components have recently been demonstrated to be under the control of independent gene(s) and capable of partitioning (uncoupling) through recombination in neo-polyploid apomicts[5]. Their independent inheritance has also been demonstrated along with the potential that apomixis phenotype can be reconstituted when individual components are combined into the same genetic background. It was also discovered that apomictic and sexual reproduction are non-mutually exclusive and represented by closely related developmental pathways which rely on deregulation of the timing of reproductive events rather than on the alteration of a specific component of the reproductive pathway[1].

Comparative genomics (structural and transcriptomics) between apomictic and sexual forms had generated important insights into the identification and expression of key genetic elements involved in reproduction forms[5]. This has enhanced the probability of success in identifying potential genes from sexual systems to generate individual mutations that mimic phenotypes of partitioned apomixis components. Disrupted functions of these genes may generate (although with variable penetrance and expressivity) unreduced female gametes (through non-reduction) as well as develop the embryo and endosperm without fertilization. A compilation of such potential genes, both from natural apomictic and sexual systems, has been extensively presented in recent reviews[6,7]. A combination of these individual mutants was successful in generating apomixis-like phenotype in *Arabidopsis*, wherein meiotically unreduced egg cells were generated by *dyad* or *MiMe* mutations followed by selective elimination

of male genome from the zygote using CENH3 lines[8]. The study also successfully demonstrated that a combination of mutants exhibiting partitioned apomixis components may develop an apomictic phenotype.

Agriculture, especially in the developing countries, could be greatly benefitted by introduction of apomixis. Efforts have been made to transfer apomixis in important cereals such as rice, wheat, pearl millet, sorghum and maize[2]. We had previously advocated the suitability of rice as a model system for breeding apomixis owing to the availability of resources in terms of interspecific variability, mutant stocks and genomics data, making it suitable for plausible approaches such as introgression, induction and mutation[9]. A model was also proposed for independent induction of individual apomixis components (apomeiosis, parthenogenesis and functional endosperm development) and their subsequent reassembly following intermating between them[3,9].

Two recent reports on the induction of apomixis in rice by Gaafer *et al.*[10] and Khanday *et al.*[11] are noteworthy to discuss in the context of breeding apomixis in rice. The first report presented induction of apomixis in an Egyptian rice hybrid using colchicine mutagenesis. The authors claimed apomictic reproduction in the hybrid line based on comparison with the parental lines and other mutant siblings, largely using agronomic, phenotypic and limited biochemical traits. Successful seed development in some mutagenized pollen-sterile lines was presumed to be of apomictic origin, supported by occurrence of cytomixis and genome content stability. The findings are interesting, but lack of information on the mode of formation of unreduced female gametes, parthenogenesis and autonomous endosperm development as well as the assumption of clonality utilizing environmentally influenced markers may lead to limited acceptance of results reported in this study. However, viable seed development in some of the pollen-sterile lines suggested the possibility of autonomous endosperm development, which may be interesting to study.

Another report, suggesting wider feasibility to develop apomictic rice, appeared in a recent issue of *Nature*[11]. The authors identified *a male-expressed rice embryogenic trigger that redirected asexual reproduction through seeds* and demonstrated that ectopic expression of BABY BOOM1, a sperm cell-expressed transcription factor, in the rice egg-cell was able to induce embryo development without fertilization. BABY BOOM is a member of the AP2 family of transcription factors, capable of triggering somatic embryogenesis from vegetative tissues[12]. The transformant line containing *OsBBM1* with an egg cell-specific promoter (*pDD45 :: BBM1*; designated as BBM1-ee) showed parthenogenetic embryo development.

Subsequently, clonal reproduction in rice was obtained following the principle that apomixis *in toto* can be achieved by assembling individual partitioned apomixis components (from the respective mutants/transformants). Development of the line expressing the first apomixis component, viz. apomeiosis (formation of meiotically unreduced egg cell) relied on the identification of three genes whose combined defects converted meiosis to mitosis (MiMe; Mitosis instead of Meiosis) in *Arabidopsis*[13] and rice[14]. The triple knockout of meiotic genes, viz. *PAIR1* (abolishes meiotic recombination), *REC8* (modifies chromatid segregation) and *OSD1* (causes omission of second meiotic division) in the MiMe line producing meiotically unreduced and diploid male and female gametes were also generated by Khanday *et al.*[11].

Further, the authors developed haploid as well as diploid rice stocks containing a combination of both the referred genes, i.e. *MiMe* + *BBM1*. Interestingly, both haploid and diploid plants were recovered from the progeny of these stocks that were confirmed for asexual propagation through seeds. These plants successfully combined the capacity of unreduced gametes formation through *MiMe* and the development of parthenogenetic egg cells from *BBM1*, though with frequencies between 11% and 29% in diploids and 15% and 26% in haploids. The clonal reproduction was inheritable to several subsequent generations.

MiMe lines had the advantage that they were almost fully penetrant with absolute expressivity in generating unreduced gametes, unlike *dyad*/*swi1* mutants[15]. However, the expressivity of parthenogenesis imposed by *OsBBM1* was limited (maximum 29%). This suggested, as in natural systems, that parthenogenesis may require contribution/regulation of additional factors such as modifiers, epigenetic regulation or polyploidy[16]. We have also recently demonstrated that increase in ploidy level greatly enhances the expression of parthenogenesis, but its effect on the apomeiosis component was negligible[17].

Importantly, as *MiMe* and *BBM* genes are largely conserved across the plant kingdom[11,13], they also offer advantage to devise similar strategies to achieve synthetic apomixis in a variety of agriculture and horticulture crops. Indeed the potential of *BBM-Like* gene from *Pennisetum squamulatum* (*PsBBML*), a wild relative of pearl millet, has recently been demonstrated to induce parthenogenesis in pearl millet, rice and maize[18]. Other potential genes capable of generating similar phenotypes include *BELL1* (a master regulator for gametophyte-to-sporophyte transition capable of triggering embryogenesis and asexual reproduction in mosses)[19] and CENH3 stocks (altered centromeric histone variant deriving elimination of haploid genome)[20].

This note has also established the importance of developing mutant stocks spanning the maximum possible genomic regions and supported the view that the dynamics of seed development in plants can specifically be altered with a combination of suitable mutants. In fact, all the major genes affecting meiotic recombination, progression of cell division, fertilization, embryogenesis and subsequently endosperm development[5,7], have the potential to generate an altered reproductive phenotype.

Since the endosperm is the most economical tissue, it becomes imperative to ensure that its development remains normal, and its nutritional and physiological characteristics are sustained. It was interesting to observe parental genome doses in the endosperm of synthetic apomictic lines reported by Khanday *et al.*[11]. Haploid and diploid synthetic apomicts showed 2maternal : 1paternal genome contribution in endosperm similar to normal sexually generated seeds, even though the embryo : endosperm ploidy ratios differed from 2 : 3 in sexual plants to 1 : 3 and 2 : 6 in haploid and diploid synthetic apomicts respectively. This also suggests that, otherwise strictly regulated, endosperm imprinting is relaxed in these mutants possibly by virtue of embryological development or pleiotropic effect of any/some of these mutant alleles.

Some notable observations in the present study which may yield future directions for generating perfect apomixis system include enhancing the frequency of parthenogenesis, targeted cell-to-cell signalling, identifying genes inducing unreduced gametogenesis only in females and minimizing endosperm imprinting effects specifically on endosperm development and starch mobilization. Although a two-component system has been used in developing apomictic rice[11], and was also advocated by Conner and Ozias-Akins[2], we suggest utilizing a

three-gene strategy (one each for female apomeiosis, parthenogenesis and endosperm development) by including additional genomic factors leading to autonomous endosperm development (development of endosperm without fertilization) in a male-sterile background, which may allow successful development of seed while minimizing the chances of escape of 'apomixis' or its components through pollen-mediated gene flow[21]. Candidate genes for autonomous endosperm development are being extensively studied for their potential utilization[7,22]. Introducing poly-embryony in apomictic rice, utilizing genes such as *OsPE1* from rice[23], other cereals or citrus[24] would be an added advantage and deserve attention from apomixis researchers.

Interestingly, some of these approaches to generate apomixis in cereal crops, including rice, have already been proposed in at least three of the 16 articles on apomixis that were published in *Current Science* during 1964 to 2019 (refs 3, 9, 25). Correctly foreseen, these articles emphasized modification in the cell-cycle genes[25] to generate individual apomixis components followed by their reassembly[3,9] to generate the apomixis phenotype. It was stated '...following these approaches, if an individual component is produced, apomixis can be obtained as a whole by recombination events…' with special reference to rice[9], and '...efforts for producing apomictic cereal may be strengthened by inducing individual components and then obtaining recombinants between them in order to obtain apomixis in toto…' as a generalized model for other crops[3].

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