

# Prospects for breeding apomictic rice: A reassessment

P. Kaushal\*, D. R. Malaviya and A. K. Roy

*Rice, being the most important cereal, attracted major attention in genomics as it offers advantages of having the smallest and evolutionary ancestral cereal genome. Its genomic status, recent discoveries of highly efficient screening procedures such as Flow Cytometric Seed Screen for identification of apomixis (or its components, viz. apomeiosis and parthenogenesis), as well as synteny of molecular markers for apomixis added to the advantages of this system. Rice appears to be an ideal model system for apomixis breeding following all the three approaches that include (i) introgression of apomixis (or its components) from related Poaceae species or its induction following Carman's Hybridization Derived Floral Asynchrony hypothesis, (ii) mutagenesis and (iii) molecular biology and genetic engineering approaches. Recent progress on molecular markers for apomixis, genetic mapping and their synteny with rice provides a strong platform for accelerating attempts to produce apomictic rice. A reassessment for prospects of breeding for apomictic rice is discussed.*

APOMIXIS research has gained wide importance in the last two decades due to its potential to revolutionize global agriculture<sup>1</sup>. The prospect of introducing apomixis into cereal crops may even dwarf the Green Revolution and therefore apomixis was termed 'asexual revolution'<sup>2</sup>. Potential advantages of apomixis breeding were widely discussed<sup>3-7</sup>. Cellular and molecular mechanisms involved in the expression of apomixis were widely discussed and several models proposed to explain the origin, evolution and inheritance of apomixis<sup>1,8-14</sup>.

Attempts to produce apomictic cereals have had some success, but an apomictic cereal variety is still awaited. Attempts are being made in pearl millet (USDA-ARS, Tifton) and in maize (CIMMYT, Mexico) to introduce apomixis from wild relatives (*Pennisetum squamulatum* and *Tripsacum dactyloides* respectively) through introgression. Some efforts were also made in wheat<sup>15</sup> and sorghum<sup>16</sup>. Amongst cereals, pearl millet and maize offer advantages for apomixis breeding, since related wild species are available from which apomixis trait can be transferred using wide hybridization strategies. Extensive research attempts made on these crops since the last two decades have produced significant results<sup>17,18</sup>. However, success in obtaining a true apomictic cereal of commercial importance was limited since endosperm development is often restricted. Marker-assisted breeding, map-based cloning and other approaches followed in these crops reveal the genetic regulation of apomixis and identification of the key genes involved in the process<sup>18,19</sup>. A suitable sys-

tem that interferes least with endosperm development and regulation in such approaches is needed for introducing apomixis in cereal crops. One approach being followed to overcome such problems is to insert a transposon in the alien addition lines in these species to induce breaks in the chromosome region harbouring apomictic genes, leading to its transfer into commercial cultivar parents.

Apomixis breeding on rice has attracted attention since long<sup>20-23</sup>, as rice research has one of the biggest networks worldwide. Many laboratories around the world are involved in rice apomixis research<sup>5</sup>, including IRRI Philippines; CSIRO Australia; CAMBIA and USDA. Although some Chinese laboratories published their results on the production of apomictic rice<sup>24-27</sup>, these received limited international acceptance<sup>28-31</sup>.

There are several reasons which make rice one of the best systems to introduce/induce apomixis. Rice is a model plant for genetic and breeding research on account of its small genome size (430 Mb), highly dense molecular map (4000 DNA markers), YAC, BAC, EST libraries, TDNA insertion and deletion mutants, gene bank with 100,000 accessions, several wild species, good transformation protocols and above all a huge database on genome sequences of japonica and indica rices<sup>32-35</sup> (<http://ars-genome.cornell.edu/rice>, <http://www.gp.dna.affrc.go.jp>). We present here an update on apomixis research which favours rice as a potential crop for induction of apomixis.

## Approaches for apomixis-breeding and suitability of rice

Three approaches are presently being followed in apomixis breeding: (a) introgression, viz. transfer of apomixis gene(s)

The authors are in the Crop Improvement Division, Indian Grassland and Fodder Research Institute, Jhansi 284 003, India

\*For correspondence. (pkaushal@rediffmail.com)

from wild relatives through interspecific hybridizations; (b) mutagenesis and (c) molecular biology and genetic engineering approaches for induction or *de novo* synthesis of apomixis. Critical studies on the induction of apomixis<sup>6,36</sup> show that rice is perhaps one of the best cereals for apomixis breeding, since it is amenable to all the experimental approaches towards production of an apomictic cereal<sup>21</sup>.

### *Introgression: screening for individual components and HFA prospects*

This approach appears most plausible as it allows introgression of apomixis gene(s) once F1 is produced and followed by selections in back-cross generations. It is being followed in pearl millet and maize–*Tripsacum* apomixis breeding programmes where wild apomictic relatives are available<sup>19,37,38</sup>. Wild and cultivated germplasm of rice were screened for apomixis, but no apomictic accession could be identified<sup>20,21,39,40</sup>, although occurrence of twin seedlings<sup>41,42</sup> and multipistillate ovaries<sup>43</sup> was reported. The limited number of accessions/species tested for apomixis could be one of the reasons for inability to find apomictic forms. *Oryza* species are so widespread and diverse that obtaining apomictic types should not be a problem. Screening methods used to identify apomixis in such studies included histological, pistil clearing and/or callose deposition test<sup>21,39</sup>. These procedures, although relatively rapid, still suffer from limitations of technical expertise and time. Moreover, none of these techniques account for apomixis as a whole, but only a single component of apomixis, i.e. apomeiosis (apospory/diplospory) could be identified. Apomixis has two major components, viz. apomeiosis (leading to formation of unreduced embryo sac) and parthenogenesis (fertilization-independent development of embryo). Both are necessary for apomixis to occur<sup>44</sup>. The above techniques verify the first component but fail to account for the second. The absence of parthenogenesis in ovules with unreduced embryo-sac formation often results in B<sub>III</sub> plants in the progeny of apomictic individuals<sup>45,46</sup>. Thus, it becomes essential to verify both components of apomixis. Occurrence of even a single component is important, as attempts can be made to introduce the (genes for) other component by hybridization. Recently, a novel method for identification and quantification of apomixis was provided and termed as Flow Cytometric Seed Screen (FCSS)<sup>45</sup>, which accounts for both the components. FCSS can reconstruct the reproductive pathway of seed development by measuring relative DNA contents from embryo and endosperm cells of mature seeds. This technique, used in many agamic crops, is reported to work in rice<sup>45</sup>. The use of FCSS for identification of apomixis in *Oryza* germplasm is expected to yield favourable results with sections known to exhibit higher proportion of disturbed or abnormal sexual behaviour, such as adventitious embryony, twin seedlings, etc.<sup>41,47</sup>. Additionally, as no ideal apomictic species has appeared so far in Oryzoidae, other

sub-families or families can be studied. Efforts to produce interspecific hybrids of rice with *Cenchrus ciliaris*<sup>48</sup>, *Penisetum alopecuroides*<sup>49</sup> and *Panicum maximum*<sup>50</sup> following sexual crosses (utilizing embryo rescue) or protoplast fusion were successful.

Hybridization-derived Floral Asynchrony (HFA) can be tried for apomixis induction, as suggested by Carman<sup>9</sup>. Apomixis, as a form of reproduction, can be obtained following HFA, when two distinct sexual ecotypes from diverse geographical (environmental) locations differing in their meiotic capabilities are hybridized. Similar genome (AA) or even dissimilar genome of *Oryza* species must be included in such efforts. Formation of restitution nuclei was reported in F1 hybrids between *O. sativa* and *O. officinalis*<sup>51,52</sup>. Interspecific hybridization in rice was utilized for crop improvement using information on inter-specific compatibility<sup>53–55</sup>.

### *Induction of apomixis through mutagenesis*

Mutagenesis is another approach for induction of apomixis in rice that holds promise in view of availability of diverse mutation stocks. A combination of effective mutation method along with good screening procedure such as FCSS is useful to identify complete apomixis or its component traits. Panicle and embryo mutants in rice were earlier reported<sup>21</sup>. Genes for parthenogenesis (second component of apomixis) may be searched in genotypes (or mutants) that exhibit high frequency of haploid formation, similar to the Salmon system in wheat<sup>56</sup>. Also, information on *Arabidopsis* reproductive mutants such as *fie*, *fis* and *mea* that exhibit some aspects of apomixis<sup>13,57</sup> needs to be considered. Once such genes are known to induce apomixis or its components, perhaps rice will be the first choice for transformation experiments. Genes involved in female gametophyte development could be an additional target for such manipulation for which rice is ideal. Isolation of genes from rice homologous to *ovm2* (from *Arabidopsis*) and *mei* (from maize) is important in understanding gene expression during female organogenesis.

### *Molecular biology approaches for induction of apomixis in rice: the promises*

Most encouraging is the induction of apomixis through molecular biology approaches, based on recent discoveries on genomics in rice. Two reports are of interest. First, the discovery that rice contains the ancestral genome in the family Graminae and that all the maps of Graminae could be combined into a single integrated grass map with rice as its base genome<sup>58</sup>. Rice has the smallest genome amongst cereals, for which the densest maps and tools for genomic analysis are available<sup>59</sup>. Rice genome is proposed as a base for evolution of grasses that contain blocks of linkage segments, giving rise to chromosomes of other Graminae species. Such a phenomenon has stimulated

interest in rice for identification and characterization of genes for major agronomical traits, even though they might not be present per se<sup>60</sup>.

Secondly, the discovery of synteny of genes<sup>61</sup> for important traits within the family Graminae and rice is encouraging. Pupilli *et al.*<sup>62</sup> discovered that the chromosome segment related to apomixis in *Paspalum simplex* is homeologous to the telomeric region of the long arm of rice chromosome 12. Five rice RFLP clones were identified showing absolute (100%) linkage with apomixis that mapped spanning 14.5 cm in the telomeric part of rice chromosome 12, but clustered together in the corresponding *P. simplex* area, exhibiting a strong restriction of recombination. It is logical to believe that factors that trigger the apomictic process may be found in such apomixis-specific chromosome segments<sup>62,63</sup>. The discovery of gene sequences present in a sexual crop (rice) as well as in an apomict (*P. simplex*) is informative. Such sequences are considered as 'hot spots' for induction of apomixis via genetic engineering. It may be of help to directly induce apomixis in rice engineering in this chromosomal area at the structural and functional levels. Such an effort could trigger apomixis or some of its components. Induced polyploidy may also be coupled at this stage to supplement the higher gene dosage effect for expression of apomixis. Similar results were observed on strong syntenic relation between apomixis locus and rice probes in *Paspalum malacophyllum*<sup>64</sup> and *P. notatum*<sup>65</sup>, as well as with ASGR (Apospory Specific Genomic Region) of *Pennisetum squamulatum* and *Cenchrus ciliaris*<sup>66</sup>. These discoveries may not only supplement but accelerate attempts to produce apomictic rice. One of the possibilities could be to utilize molecular biological tools for characterization of such sequences. Since apomixis is a modification of the sexual developmental process, mostly affecting the regulation of meiosis or the tuning of megagametogenesis<sup>11,13,19</sup>, the regulation of a function that is most likely highly conserved across plant species shall be the target. Additionally, it is possible that genes for apomixis present in apomicts are also probably present in their sexual relatives and thus have some relic counterpart in rice.

Characterization of such sequences as well as syntenic relationships in Graminae are important for searching genes that play a key role during apomixis. Recently, Yamada and Kishida<sup>67</sup> observed synteny of many rice RFLP markers in grasses like *Lolium* and *Festuca*. Studies on synteny of molecular markers for apomixis in agamic complexes such as *Pennisetum*, *Paspalum*, *Panicum*, *Brachiaria* and *Tripsacum* with rice are useful<sup>62,64-66</sup>. Our results on syntenic relationships of one of these markers (C1069) across the Graminae family were highly encouraging as similar sequences were observed in other agamic species belonging to the genera *Poa*, *Paspalum*, *Pennisetum* and *Panicum* (Kaushal, Matzk and Baumlein, unpublished). Related sequences may be identified and should be the target for genetic manipulations. Genetic maps of the apo-regions of

*Tripsacum*, *Pennisetum* and *Brachiaria* and homologous sexual segments were compared<sup>68-70</sup>. Information obtained from recent advances in grass genomes also could be extended to rice for better understanding of apomixis. If components of apomixis can be mapped to the grass genome, then a suitable candidate should emerge from the rice database<sup>71</sup>. This approach should combine both genetics and genomics. Extension of such maps to rice is of great importance. Now that several molecular markers for apomixis have been identified<sup>72</sup>, targetted mutagenesis following induced chromosomal rearrangements may help in obtaining at least some components of apomixis.

Discovery of strong synteny of apomixis-specific genomic segments from several agamic species with rice also sheds light on the possible evolution of apomixis. During evolution, the genome content might have increased<sup>61</sup> mostly by accumulation of retro-transposons and similar activities<sup>73-75</sup>. Such activities supported by chromosomal rearrangements (gross as well as cryptic) caused mutations to occur, giving rise to components of apomixis. The relationship between evolution of apomixis and genome size was earlier established and retro-transposition effects as one of the driving forces for apomixis evolution were presented<sup>74,75</sup>. Following these approaches, if an individual component is produced, apomixis can be obtained as a whole by recombination events. Independent inheritance of the components of apomixis was reported in many crops such as *Taraxacum*, *Erigeron*, *Hypericum*, *Poa*, etc.<sup>7,76,77</sup>.

Another important molecular approach followed at IRRI and other centres is to develop asexual embryos in the nucellus of rice and elimination of sexual embryos. The aim is to isolate genes capable of inducing adventitious embryony and rice promoters suitable for directing expression of these genes specifically in the nucellus during fertilization. Although genes capable of inducing embryogenesis are currently unknown, *LEC1*-like and *PKL*-like genes, which are involved in controlling embryogenesis in *Arabidopsis* were isolated<sup>78,79</sup>. Another approach is to create a synthetic form of inducible apomixis rather than obligate or facultative as found in natural apomicts, i.e. to isolate genes that can induce an apomictic embryo in the hybrid nucellus and ablate the sexual embryo<sup>23</sup>, for which a switch triggered during meiosis is essential. One such candidate gene/promoter was earlier identified<sup>80</sup>. These authors identified *DMC1* genes that were differentially expressed during meiosis, and haploid and diploid mitosis. Similar promoter::reporter gene fusions in rice were tested to determine the conserved promoter elements conferring meiosis-specific element that would have immediate application for achieving apomixis in hybrid rice<sup>80</sup>.

Lastly, one of the major problems in apomixis breeding in cereal crops is the abnormality associated with endosperm development<sup>81,82</sup>, especially where the endosperm is the most economic component of the seed. This problem is recently addressed to understand imprinting and cellular developments of endosperm formation<sup>10,82</sup>.

Extension of such results to rice might overcome problems associated with endosperm development, arising due to sexual hybridization approaches (as in pearl millet and maize), a field where rice clearly outyields its potential for apomixis breeding compared to pearl millet and maize.

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