Apomixis research in India: past efforts and future strategies

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Apomixis, a genetic method for clonal propagation through seeds, offers tremendous advantages for agriculture, especially in developing countries. Despite the promises that apomixis technology holds, research efforts in India have been limited. Our strengths combining wide biodiversity especially in the grass family, coupled with latest technological developments in the field shall redefine efforts on apomixis research. Past efforts and future directions including novel model systems and approaches to take up apomixis research in India are presented.

Keywords: Apomixis, developing countries, *Dicanthium*, future strategies, guinea grass.

APOMIXIS is a reproductive process that bypasses female meiosis and fertilization to produce embryos identical to the maternal parent[1]. It is an asexual mode of reproduction enabling clonal propagation of maternal genotype through seeds[2]. The potential advantages of apomixis when introduced into agricultural crops, particularly in developing countries, dependent on low-input cropping methods, have been widely discussed[2–4]. In a recent review, Bicknell and Koltunow[5] highlighted the advantages of apomixis technology with immediate benefits to developing countries, '...for farmers in the developing world, the greatest benefits are expected to relate to the breeding of robust, high-yielding varieties for specific environments, improvements in the security of food supply, and greater autonomy over variety ownership...'. Wide emphasis was laid in lead lectures on combining heterosis with apomixis in almost every important agricultural crop in a recently organized National Symposium on 'Harnessing heterosis in crop plants' at Indian Institute for Vegetable Research (IIVR), Banaras, during 13–15 March 2004, organized by Indian Council for Agricultural Research (ICAR) and Indian Society of Vegetable Science. Special mention of introducing apomixis technology as an 'innovative approach for hybrid development' was also made. In spite of the tremendous advantages, exceeding that of the Green Revolution, that apomixis may offer[6], research efforts have been limited in India. Being a developing country, as well as with the prevalent agriculture economy with numerous commercial hybrids getting popular, serious efforts to undertake apomixis research are urgently required. In the present article, an overview of previous apomixis research efforts made in India, potential fields of apomixis research, and future strategies to strengthen apomixis research for basic understanding and applied benefits are presented.

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Past efforts and present status

Apomixis research in India has been targetted on two strategies: inducing apomixis in cereals such as sorghum, to harness benefits of heterosis, and disrupting apomixis in forage grasses to enable effective breeding strategy through hybridization. For this purpose, identification or induction of sexuality in apomictic species was attempted. Whatever may be the target, understanding the basic mechanism of apomixis is a prerequisite, for which attempts have been limited in the past.

Past efforts in apomixis research in India have been made to selected crops like *Dicanthium* and *Sorghum*. These included experiments on apomixis expression in varied environments in former species[7,8] and isolation and characterization of few 'apomictic forms' in the latter[9,10]. It is however, important to note that these reports appeared between 1970 and 1990, and presently no publication is available on the recent status of the material generated. Guinea grass (*Panicum maximum*) is another important crop, wherein both apomictic and sexual types have been reported. Sexual forms in this crop are being utilized for production of high-yielding forage varieties using elite apomictic forms as male parents. Major efforts are being made at Punjab Agricultural University (PAU), Ludhiana and Indian Grassland and Fodder Research Institute (IGFRI), Jhansi under All India Coordinated Research Project on Fodder Crops (AICRP-FC). However, albeit an important crop with apomixis and sexuality residing in the same species and stabilized breeding procedures, basic studies on apomixis in guinea grass has not been taken up, except for limited comparisons for morphological and isozymic parameters between apomictic and sexual genotypes[11] and intergeneric hybridization[12]. Identification of sexual types in prevalent apomictic crops such as *Cenchrus*[13], *P. maximum* and *Dicanthium* is important to allow recombination and production of new varieties, and increas-

ing the gene pool of the forage species that reproduce mainly through apomixis.

Efforts on apomixis research in the past might also be limited because of the highly complex nature of the trait – as it is a modification of reproductive process, and hence, all the factors involved in reproduction require attention. However, a better understanding of the apomixis phenomenon resulting from concerted international efforts has started to unveil the complexity of the trait, and information on genetic control and molecular biology of the phenomenon has started accumulating since the last decade[5,14,15]. Recent research proposals combining present state of knowledge and crops of national interest have started attracting sponsorships from agencies such as Indian Council of Agricultural Research, Department of Science and Technology, Department of Biotechnology, etc. A *POLYCOMB* group gene of rice (*OsiEZ1*) preferentially expressed during reproductive development has been identified[16] in an ICAR-supported collaborative project between ICGEB, New Delhi and IGFRI, Jhansi on 'Identifying genes controlling cell cycle and apomixis for crop improvement'. Similarly, two *DMC1* genes important during homologous recombination in meiotic cell divisions in rice have been cloned and characterized[17]. Sexual lines in predominantly apomictic species such as *P. maximum* and *Dicanthium annulatum* were identified at IGFRI in an ICAR supported project on 'Screening for sexual lines and their characterization in apomictic perennial grasses'. Similarly, reproductive mode of seed development, validation of molecular markers in grasses such as *Poa*, *Papsalum*, *Panicum* and *Pennisetum*, and evolution of apomixis in Poaceae were reported recently[18–20] in a DST-sponsored BOYSCAST fellowship programme on 'Fingerprinting mode of reproduction and development of molecular markers for apomixis' between IGFRI and IPK, Gaterslaben, Germany. Another proposal on molecular characterization of genomic sequences involved in apomixis has been approved for financial support from ICAR and DST. Attempts are also being made for production of perennial apomictic hybrid *Pennisetum* utilizing wide hybridization between polyploid apomictic wild species and induced tetraploid pearl millet[21]. Backcross (BC_2) generation from *P. glaucum* ($2n = 4x = 28$) and *P. squamulatum* ($2n = 56$) has been produced, segregating for mode of reproduction.

Although apomixis research in India has attracted limited attention from various sponsoring agencies, important information of basic, strategic and applied research interests has been generated. Relevant information on important plant material is being generated. However, experimenting complex traits like apomixis requires more concerted and defined efforts, and hence, needs additional emphasis. Concerted efforts to identify key genes involved in apomixis in crops important in the Indian context are yet to come. Maheshwari *et al.*[22] have reviewed the potential involvement of genetic engineering to manipulate apomixis for crop improvement. Kaushal *et al.*[23] reviewed the prospects of breeding apomictic rice utilizing recent information available on molecular biology of apomixis and genetic engineering techniques.

Apomixis research in India: future strategies

We visualize a better future for apomixis research in India, because the promises that apomixis technology holds in the era of hybrid varieties in countries like India have started attracting research attention, making a platform for concerted research efforts. Secondly, international knowledge leading to better understanding of the phenomenon has been equipped with materials and procedures to handle the trait more scientifically. Information such as partitioning apomixis into component traits (viz. apomeiosis, parthenogenesis and functional endosperm development)[24], identification of molecular markers for these traits and development of molecular maps[25], and highly efficient screening procedures to discriminate sexual and apomictic types[26,27] are being utilized in the study of agamic systems already available in India.

Systematic studies involving recent molecular biology techniques on previously characterized germplasm should be a potential field to gear up apomixis research. Meanwhile, we should not neglect the natural wealth of agamic germplasm already available and maintained in the country. The role of national institutes involved in research on grasses becomes highly demanding since most of the tropical grasses are apomicts by nature and hence represent best systems to undertake apomixis research compared to other families. Institutions like IGFRI maintain the richest and most diverse but untapped source of genes for apomixis. Present gene bank collections include 28 diverse grasses, majority of them breeding through apomixis[21]. It is one of our greatest strengths, as it is the representative of all the diversity in genes controlling apomixis as well as the biological phenomenon that had occurred in the past leading to evolution of apomixis. An exhaustive characterization of germplasm base, especially the unexplored taxa, is expected to yield novel information and contrasting material for mode of reproduction that may lead to identification of new model systems unexplored till now.

Compared to grasses, collection and maintenance of dicot apomicts are limited and scattered, though some dicots such as *Hypericum* and *Hieraceum* are excellent model systems for apomixis research[28,29]. Such species, including mango and citrus fruits, representing apomixis (adventitive embryony) are also potential systems to undertake apomixis research and need to be explored. Similarly, investigating the sexual system of reproduction is important in model crops like *Arabidopsis*, *Petunia* and rice[30]. As is well understood now that apomixis is a modification of sexual reproduction[31,32], information derived from understanding sexual systems is of potential importance in apomixis research. Similarly, reproductive mutants with modifications in sexual pathway, such as *fie* and *fis* class of *Arabidopsis* mutants, are excellent materials for under-

standing molecular biology of at least some of the aspects of apomixis. Research institutions involved in basic research on genetics and molecular biology of reproduction may target similar or derived experiments.

Current apomixis research relies on two complementary strategies: 'evaluation' of the trait in natural apomictic systems, and 'synthesis' of the trait through direct modification of reproductive events in a sexual species[5]. In addition to obligate sexual and obligate apomicts, facultativeness may also be advantageous to take up the above-mentioned approaches. A review of the literature from Indian laboratories concludes that we have viable systems to take up both approaches. For these purposes, model systems such as *P. maximum* and *Cenchrus ciliaris* should be excellent, where sexuality and apomixis coexist in same species. Apomixis in *C. ciliaris* has been widely explored internationally[33,34]. *Dicanthium* could be another important system by virtue of its unique mode of development and fertilization of female gametophyte[35]. Similarly, *Pennisetum* agamic complex is another highly potential system to undertake apomixis research, a field already subjected to intense investigations at the international level[33,36]. To undertake 'introgression' approach for transfer of apomixis from wild apomictic *Pennisetum* species to cultivated pearl millet (*P. glaucum*), Indian reports are available on incompatibility reactions involved in interspecific crosses[37–39]. Interspecific hybrids between pearl millet and wild species such as *P. orientale* and *P. squamulatum* have been reported[40,41]. The progenies segregating for mode of reproduction (apomictic vs sexual) are excellent sources for 'evaluation' approach described previously, to identify key genes involved in apomixis utilizing structural and functional genomics. Most interestingly, the hybrids between sexual *P. glaucum* ($2n = 14$) and sexual *P. orientale* ($2n = 18$) have yielded triploid progenies in first and second BC generations, possibly arising from fertilization of unreduced embryo sac (B_{III} hybrids)[40]. It is important to characterize these progenies and find an explanation for *de novo* appearance of at least one component of apomixis (i.e. apomeiosis – leading to the formation of unreduced embryo sacs), as both the parental species were sexual! The hybrids and BC progenies are still maintained. If the hypothesis is proved, this might be one of the rare examples supporting Carman's HFA (hybridization derived floral asynchrony) hypothesis for evolution and synthesis of apomixis[42], a theory whose application is capable of tremendous impact on apomixis research.

With the existing wealth of germplasm, technical expertise in the related field and support due to rapidly growing understanding of the apomixis phenomenon due to international efforts, we hereby propose a five-point programme to be taken up to strengthen apomixis research in India:

1. Selection of model systems. Available diversity in agamic complexes offers better opportunities for selection of model systems, specifically for the Indian situation. Systems such as *P. maximum*, *C. ciliaris*, and *Pennisetum* are better in comparative approaches for characterizing apomixis, as both sexual and apomictic forms are available within the same species/genus and hybridization is possible. Novel systems may be searched in the germplasm. Similarly, grass germplasm holding being maintained in India is required to be thoroughly searched for identification of novel agamic complexes to be undertaken as model systems. Such unexplored taxon may enrich the variability for 'apomixis genes' in terms of structural and functional parameters. Grass genera such as *Brachiaria, Sehima, Heteropogon, Chrysopogon*, etc. are some of the potential candidates. Relying on the fact that apomixis evolution is coupled with polyploidy, special emphasis may be laid on species with cytotypes representing varying ploidy (such as *Pennisetum pedicellatum*, where tetraploid, hexaploid and octoploid cytotypes are available).

2. Effective partitioning of apomixis. Instead of dealing with apomixis as a single complex trait, it could be partitioned into its component traits (viz. apomeiosis, parthenogenesis and functional endosperm development) for advanced analysis, as demonstrated in several crops such as *Poa*, *Paspalum*, *Taraxacum*, *Tripsacum*, *Hypericum*, etc. Plant material exhibiting individual components may then be investigated for its structural, functional and regulatory aspects for identification of key genes involved in the process. Highly effective screening procedures such as flow cytometric seed screen[26], auxin test[43], callose deposition test[44], transgenesis[45], etc. are now available to characterize for mode of reproduction. 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*[23].

3. Production of alien chromosome addition lines. Cytogenetical methods may be utilized to produce alien chromosome addition lines (such as monosomic alien addition lines) for the chromosomes carrying 'genes' for apomixis from wild to cultivated species. Single chromosome is reported to confer apomixis in *P. squamulatum*[46], *Beta corolliflora*[47] and *Taraxacum officinale*[48]. Identification of the chromosome carrying apomixis genes, and its transfer into cultivated species utilizing cytogenetic stocks or chromosome engineering techniques may generate plant material directly available for commercial testing of such derived apomicts. Additionally, such lines may not pose problems of perpetuation, as they are likely to reproduce thorough apomixis. However, in such efforts suitable consideration is required to be addressed for endosperm and other related developmental abnormalities[35].

4. Evolution of apomixes. Studies on evolution of apomixis in agamic complexes could yield relevant information to understand the drift from sexual to apomictic mode, and are required to be taken up. More specifically, genera

including both apomictic and sexual species, as well as those species with different ploidy status, such as *Pennisetum* and *Panicum* may be a system of choice. The effects of hybridization, polyploidy and ecological adaptations[49] on evolution of apomixis are still unclear. Relation of apomixis with increased haploid genome size[20], point of origin of apomixis in evolutionary tree of family Poaceae, role of retrotransposition effects, etc. are some unanswered questions. Experiments are required to be undertaken on such evolutionary studies, for which systems such as *Pennisetum*, *Panicum*, *Brachiaria*, *Paspalum* and *Taraxacum* are ideal, where the genus is represented by apomictic as well as sexual species.

5. Exploring synteny of regions governing apomixes. The discovery that apomixis-specific sequences are represented in cereals offers a scope for genetic engineering in apomixis[15,46,50]. Search for molecular markers/genomic regions involved in apomixis validated in diverse agamic and sexual systems is necessary for identifying key (or regulatory) sequences. Validation of known molecular markers for apomixis into Indian germplasm, identification of sequences involved in apomixis (or its components) expression utilizing approaches such as representational differential analysis (RDA), subtractive hybridization, etc. followed with utilization of bioinformatics to annotate functions to these sequences may identify the key genes involved and possible mechanism of their interaction. Furthermore, information that apomixis is a deregulated sexual pathway of reproduction with modifications at least at two points, viz. meiotic development of female gametophyte and fertilization event[5,44], it becomes essential to identify and explore the contribution of regulatory sequences and their similarity across different agamic groups. Modification/engineering in such regions offer advantages to understand and manipulate the phenomenon. Equally important is to understand the phenomenon of sexual development. Identification of key genes involved in sexual reproductive development[16,17,22] to identify differences with and apomictic pathway and their regulation may be informative in understanding the molecular mechanism of apomictic mode of reproduction.

In view of the above strategies that rely on identification and/or generation of appropriate plant materials to study apomixis and its components, involving modern biotechnological tools, concerted efforts are required to be taken up (as proposed in Figure 1) utilizing synergy between plant breeders, geneticists and molecular biologists to identify and characterize genes involved in apomixis for its effective utilization in crop improvement in India.

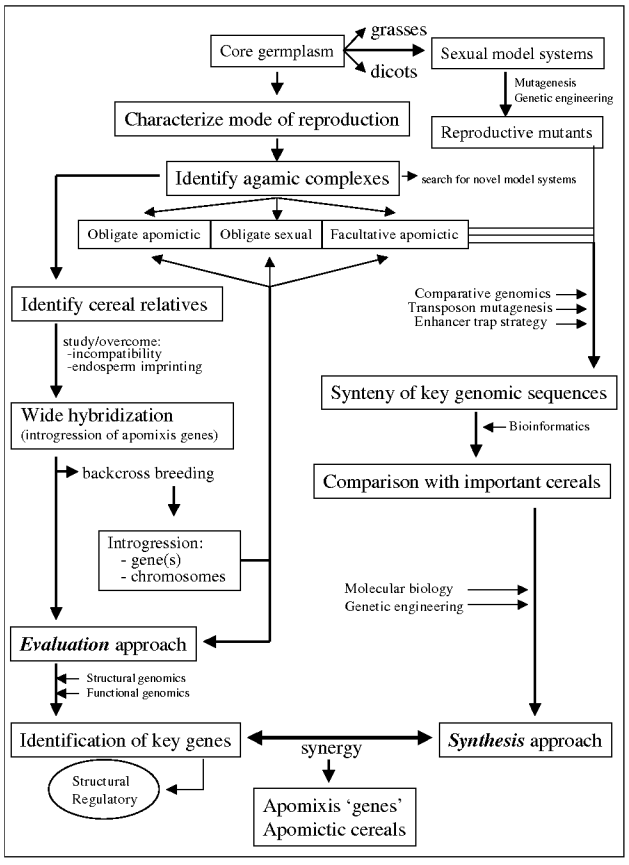

Figure 1. Schematic proposal for a model of apomixis research in India to utilize 'evaluation' and 'synthesis' approaches for identification and utilization of relevant apomixis genes.

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