

Rajesh M. K., K.K. Sajini, K.S. Muralikrishna and Anitha Karun

Division of Crop Improvement, ICAR-Central Plantation Crops Research Institute, Kasaragod 671124, Kerala.

The coconut palm (Cocos nucifera L.), eulogized as 'Kalpa Vriksha', is quite extraordinary for its use in the manufacture of various products and as a provider of direct livelihood for millions of people in the tropical coconut growing regions of the world, in addition to its contribution to environmental sustainability in these fragile ecosystems. Conventional coconut breeding is quite cumbersome given the perennial nature of the palm, its sizeable phenotypic diversity, low multiplication rate, highly heterozygous nature and lack of means for vegetative propagation. The use of biotechnological tools has permitted researchers to overcome some of these difficulties associated with conventional coconut breeding.

Embryo culture and embryo rescue

In coconut, production of planting material for propagation is exclusively through seed nuts. Some of the major limitations come across during collection, transport and storage of coconut germplasm as seed nuts include the bulkiness of the seed nut, its short dormancy period, presence of nut water, stringent phytosanitary requirements, increased risk of pests and diseases and huge cost for transportation. The



Fig. 1. Embryo rescue of sweet kernelled genotypes

collection and transportation of coconut germplasm through embryos, instead of seed nuts, is recommended by FAO/IPGRI. The coconut embryo culture protocol has been standardized in ICAR-CPCRI. This technique has enabled introduction of exotic germplasm (45 accessions from eight countries) into the country. The technique also finds use for embryo rescue in coconut with special traits like coconut with sweet kernel (Fig. 1), horned coconut and Makapuno type of coconut after cryopreservation of coconut (which do not germinate naturally), for collection of rare germplasm, as well as-----

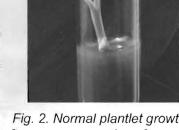


Fig. 2. Normal plantlet growth zygotic embryos

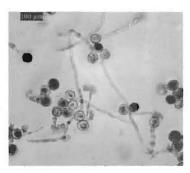


Fig. 3. Germination of cryopreserved coconut pollen

Cryopreservation for germplasm conservation

coconut zygotic embryos.

The prevalent mode of conservation of coconut genetic diversity is through establishment of field gene banks. A complementary conservation strategy has been envisaged for safe and effective conservation of entire gene pool of coconut. Cryopreservation of coconut zygotic embryos and pollen (Fig. 2 and 3) has been successfully employed at ICAR-CPCRI as an adjunct technique for long-term conservation of coconut germplasm, thereby shielding valuable genetic resources from biotic and abiotic threats. For pollen, its collection, processing and storage in liquid nitrogen have been assessed and found to be successful in terms of pollen viability and fecundity for long term storage upto a period of six years of study, which substantiates its efficacy in long term conservation. The successful preservation of somatic embryogenic cell cultures would facilitate the production of many more coconut plants from one initial explant.

for developing cryopreservation techniques utilizing

In vitro culture for rapid multiplication

A major impediment to enhancement of coconut productivity is the production and distribution of homogeneous quality planting material to the farmers. The present annual production of coconut seedlings through conventional techniques is unable to meet the annual requirement of quality planting materials. Rapid multiplication of coconut through in vitro techniques, therefore, is of paramount importance. However, recalcitrance of coconut to in vitro culture is still a major bottleneck. Plumular regions are juvenile tissues which have responded best to in vitro culture. The in vitro regeneration protocol from plumular explants has been improvised using shoot meristematic tissues excised directly from the fresh embryo. Early callus induction and significantly greater embryogenic potential and subsequent plantlet development has been achieved (12 somatic embryos/plumular tissues on an average) (Fig. 4 and 5). Even though plantlets have been regenerated and successfully established in the field, a large



Fig. 4. Embryogenic callus obtained from plumular explants of coconut



Fig. 5. Plantlets derived from somatic embryos

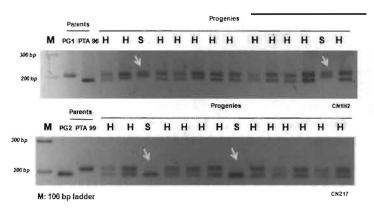


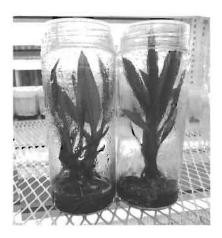
Fig. 6. Confirmation of coconut hybrids (CGD x WCT) using microsatellite markers (PG: CGD parent, PTA: WCT parent, H: Hybrids, S: Selfed progenies)

scale clonal propagation of coconut is yet to be accomplished with conversion of somatic embryos into plantlets remaining one of the major bottlenecks. It is necessary to consider and then employ procedures that are successfully used for other species to help drive future improvements in coconut in vitro culture.

Molecular markers for genetic diversity studies and marker-assisted selection

Molecular markers have the capacity to enhance breeding efficiency in coconut in distinct ways through germplasm characterization and management, linkage mapping and identification of quantitative trait loci (QTL) markers for marker-assisted selection (MAS). Origin of coconut palm had remained a puzzle for many years. Through molecular marker studies, evidences have been provided for two independent origins of coconut, in the Pacific and Indian Ocean basins. Studies, utilizing molecular markers, have revealed genetic distinctiveness of tall and dwarf coconut accessions, which can be attributed to the differences in their breeding behaviours: the self-pollinating dwarf coconut accessions display less phenotypic and genetic diversity and more homozygosity in contrast to talls, which are crosspollinated.

Markers associated with important traits can increase the breeding efficiency, thus reducing the time for a breeding cycle in a perennial crop like coconut. Molecular markers have been identified for differentiating tall (T) and dwarf (D) cultivars of coconut. These markers have also been utilized for confirming the purity of D x T hybrids which will ensure supply of genuine hybrids to farmers (Fig. 6). Selected alleles of SSR or RAPD markers have been associated with resistance to eriophyid mite and lethal



yellowing disease which would allow for selection of these traits in the seedling stage itself. QTL mapping of important traits such as early flowering, yield, fruit components and composition of cuticular wax have been identified so far. Strong QTLs and saturation of coconut linkage map with an additional set of markers can increase the breeding efficiency of coconut for the traits of interest.

Conclusion

As can be envisaged from the above, biotechnological techniques offer enormous potential for development of new useful coconut varieties, with the available diverse germplasm, by overcoming impediments associated with traditional breeding techniques. Adoption of a combination of classical breeding methods with modern biotechnological techniques will lead to the rapid improvement in coconut breeding objectives. Improvement of biotechnological protocols and their applications to a repertoire of coconut germplasm will definitely open up new prospects for collection, conservation, breeding and productivity of coconut