different pathways were identified that converge on a set of floral pathway integrator that activates flower meristem identity genes such as *LEAFY (LFY)* and *APETALA1 (AP1)*.

LFY is the first gene to be expressed amongst them and its overexpression has been found to accelerate flowering in many species. This ability of *LFY* to accelerate flowering is tapped in this study to develop early flowering transgenics of *Brassica* by overexpression of *Atleafy*. *B. juncea* cv. Geeta selected for the study has high oil content and suitable for rainfed conditions of Delhi, Punjab and Haryana and matures in 148-160 days.

To introgress *Atleafy* gene, initially a reliable and reproducible regeneration protocol was established using hypocotyl and cotyledonary leaves as explants, where cotyledonary leaf showed better regeneration than hypocotyl. MS + NAA 0.1mg/l +BA 2mg/l proved to be the most suitable medium. It induced shoot regeneration and its multiplication in 97.5% cultures of cotyledonary leaf. Rooting occurred when regenerated shoots were transferred to MS medium supplemented with IBA 2 mg/l.

MIC for the selection marker (Kanamycin) in the current study was 30mg/l. Hypocotyl and cotyledonary leaf from 5-day-old seedlings were used as explants for transformation. The explants were transfected for 7 min and then co-cultivated for 48 hr in dark for best transformation efficiency. The transformation efficiency of cotyledonary leaf was higher than the hypocotyl. Integration of the transgene in transformed plants was analysed by GUS assay. Further the transgenic nature of the plantlets was confirmed by PCR and Southern blotting.

POTENTIAL USE OF MOLECULAR AND BIOTECHNOLOGICAL TOOLS IN IMPROVEMENT OF COCONUT, ARECANUT AND COCOA

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Plantation crops form an integral part of the horticulture. Plantation crops, by nature, blend effectively with environment contributing to sustainability, conservation of bio-diversity and stable ecosystem. Although plantation crops occupy less than 2% of the total cultivated area, they are earning over Rs 3700 million annually through exports. This sector constitute a wide range of crops and among them coconut, arecanut and cocoa are important for livelihood of small and marginal farmers. They also play an important role in employment generation as well as poverty alleviation in rural India. At present, coconut, arecanut and cocoa are grown in 2.07, 0.446 million ha and 71,000ha with an annual production of 23351 million nuts, 0.608 million tones and 18,000 tonnes respectively. The recent advances in plant molecular biology and biotechnology and the achievements of research in perennial crops have shown that the potential application of these tools to plantation crops . At ICAR-CPCRI, we have concentrated our efforts on developing: 1) micropropagation protocols and cryopreservation, 2) molecular marker technologies for mapping of height of the plant, abiotic and biotic stress traits and identification of hybrids, 3) Transcriptome profiling and 4) genomics and bioinformatics. The results of these studies are presented in this paper.

Tissue Culture and Cryopreservation

Production of homogeneous quality planting material to the farmers is very important and is one of the major constraints in coconut productivity. The present annual production of coconut seedlings, through nuts, is unable to meet the annual requirement of quality planting materials. Rapid multiplication of coconut through *in vitro* techniques, therefore, assumes paramount importance. However, coconut has remained highly recalcitrant to *in vitro* culture. Tissue culture of plantation crops was initiated at ICAR- Central Plantation Crops Research Institute (CPCRI) four decades back and repeatable *in vitro* protocols have been developed for oil palm and arecanut inflorescence culture. Plumular regions are juvenile tissues which were reported as explants responding best to *in vitro* culture in coconut compared to other explants. Even though plantlets have been regenerated and successfully established in the field, a commercial scale protocol has not been achieved and conversion of somatic embryos into plantlets has remained one of the major bottlenecks. Presently, upscaling of somatic embryogenesis pathway is being attempted through the use of bioreactors.

The coconut embryo culture protocol developed at ICAR-CPCRI was instrumental for introduction of exotic germplasm (45 accessions) from Indian Ocean islands and also for embryo rescue in coconut with special traits like sweet coconut ('*Mohachao Narel*') and '*Makapuno*' type of coconut as well as for developing cryopreservation techniques utilizing coconut zygotic embryos.

For the recalcitrant seeded coconut, the present mode of conservation of genetic diversity is through field gene banks. A complementary conservation strategy involving a combination of different approaches and methods may be envisaged for the effective conservation of entire gene pool of coconut. Cryopreservation of embryos and pollen could be employed as an alternative method for long-term conservation of coconut germplasm, thereby sheltering genetic resources from biotic and abiotic threats. Four different cryopreservation methods were experimented for coconut zygotic embryos. These include air desiccation, pregrowth desiccation, encapsulation dehydration and vitrification and exhibited different degrees of success rate ranging from 10% in air desiccation to 27.5% in modified pregrowth desiccation method with respect to *ex vitro* establishment of plants in pots. But considering the operational simplicity and the equipments required the vitrification method with success rate of 22.5% can be extrapolated for routine application in gene banking. Preliminary studies involving storage effect of embryos in liquid nitrogen for successful plantlet recovery also favors this method. For pollen, its collection, processing and storage in liquid nitrogen were assessed and found to be successful in terms of pollen viability and fecundity for long term storage upto a period of 6 years of study that substantiates its efficacy in long term conservation.

Somatic embryogenesis and plantlet regeneration from inflorescence explants of arecanut was reported for the first time at ICAR-CPCRI, Kasaragod. The protocol was tested with different arecanut varieties *viz.*, Mangala, Sumangala and Mohitnagar. Clonal fidelity studies using molecular markers have revealed the genetic uniformity of the regenerated plantlets with the mother palms. Tissue cultured plantlets were field planted during 2006 at Vittal for field evaluation. This protocol was applied for mass multiplication of field resistant Yellow Leaf Disease (YLD) palms and plantlets were planted in farmer's plots in YLD 'hot spot region' at Sullia, Karnataka. Subsequently the protocol has been applied to Hirehalli Dwarf and Dwarf hybrids.

Molecular markers

Selection of hybrid seedlings in coconut and arecanut, relying on morphological parameters, often results in selection of undesirable out-crossed seedlings and also rejection of few good hybrids. Molecular markers have been identified for differentiating tall and dwarf cultivars of both coconut and arecanut. These markers have been converted as Sequence Characterized Amplified Region (SCAR) markers and validated and also utilized to authenticate hybrids in D (dwarf) x T (tall) crosses in coconut and arecanut.

DNA finger printing using microsatellite markers have been carried out in 139 coconut accessions to document the genetic integrity and diversity. A panel of SSR markers has been identified for confirming the hybridity of D x T hybrids (CGD x WCT) which will ensure supply of genuine hybrids to farmers. Sixty indigenous and exotic arecanut accessions have been analyzed using RAPD and SSR markers. The clustering pattern was, in general, in accordance with the geographical origin of the collections.

Gene expression studies

Transcriptome profiling of coconut root (wilt) disease susceptible and resistant *Chowghat Green Dwarf* cultivar using RNA-Seq revealed 266 transcripts to be up-regulated in resistant samples. Many of these differentially expressed transcripts were primarily involved in defense responses, signaling pathways, cellular transport and other metabolic processes. Transcripts induced during water-stress have been cloned and characterized using differential display RT-PCR. Based on the results obtained in this study, gene-specific primers were designed to amplify genes induced during water-stress in coconut. Resistant Gene Analogues (RGAs) were cloned and characterized from coconut using a degenerate-primer based PCR strategy and comparative genomics.

Transcriptome and proteomics studies have been initiated at CPCRI to decipher the molecular basis of somatic embryogenesis in coconut. RNA-Seq of embryogenic calli obtained from the plumule derived calli was performed on an Illumina HiSeq 2000 platform. Real-time PCR analysis of different genes expressed during somatic embryogenesis of coconut (*SERK, GST, GLP, CLV, WUS, PICKLE* and *WRKY*) was performed and differential gene expression pattern was noticed during different stages of development.

Bioinformatics

With support from Department of Information Technology (Agri-Bioinformatics Promotion Centre) and Department of Biotechnology, several comprehensive databases for the mandate crops have been developed. MAPS (Microsatellite Analysis and Prediction Software), a bio-Java based independent; stand alone platform was designed to allow the identification and characterization of microsatellites in entire genomes. Software's were developed for detection of microsatellites in whole genome sequences ('MAPS'), prediction of domains and motifs in disease resistance genes in palms ('PRGPred') and prediction of gibberellic acid biosynthetic genes in palms ('GA Pred'). Also, expert systems for 'Coconut Artificial Pollination Management' and a 'Pest and Disease Surveillance Database' have been developed. A database of microsatellite markers genotyped in coconut accessions ("CMSD') was also developed. Homology-based modeling and docking studies were undertaken to deduce the 3-D structure of proteins induced during stress in coconut (WRKY, phospholipase D, cyclic nucleotide gated channel protein, lipoxygenase), somatic embryogenesis (SERK, BABY BOOM, KNOX)

Genomics

Large quantity of availability of cocoa ESTs in public domain helped in identification of genes related to *Phytophthora* pod rot and moisture stress related genes. Cocoa genome has been sequenced and available in the public domain and access of genome resources enabled identification and reconstruction of biosynthetic pathways contributing to fatty acid and flavonoid biosynthesis as well as biotic and abiotic stress related pathways. Utilization of genomic tools will definitely provide a fillip in speeding up the traditional breeding programmes in cocoa.

Future strategies include:

- Enhancing *in vitro* regeneration in coconut.
- Genome sequence analysis in coconut.