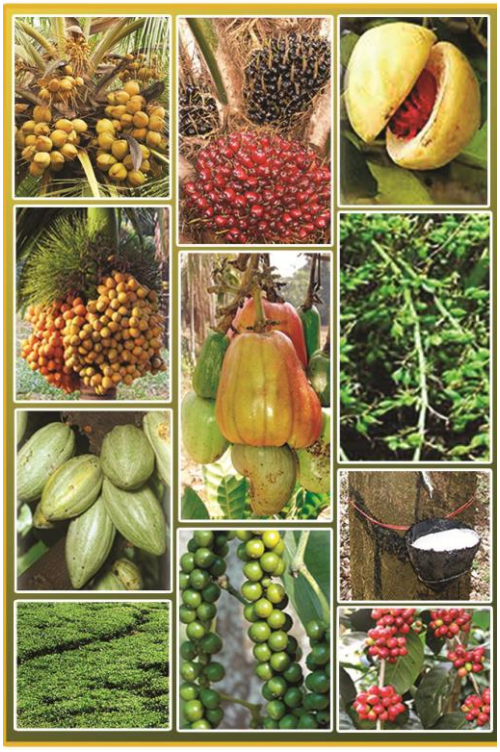
Workshop on
Breeding Strategies in Plantation Crops

27.04.2018
ICAR-CPCRI, Regional Station, Vittal

Compendium of Abstracts



ICAR-Central Plantation Crops Research Institute
Kasaragod - 671 124, Kerala

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Molecular Marker-Assisted Breeding for Crop Improvement: Drought Resistance in Rice - a Case Study

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Drought stress is a major constraint for rice production, especially in rainfed ecosystems. Landraces are reservoir of valuable traits that can help in breeding drought resilient cultivars. Utilizing the genetic potential of these locally adapted resilient germplasm using quantitative trait loci (QTL) mapping and marker-assisted breeding (MAB) will hasten development of high yielding drought resilient cultivars.

i. Mapping of consistent QTLs for plant production traits under drought in target populations of environment (TPE) using local rice lines

a) A total of 27 genomic regions linked to phenology and plant production traits under drought predominant in rainfed target populations of environment (TPE) were mapped using rice lines derived from a locally adapted landrace, Nootripathu and drought susceptible elite cultivar, IR20 evaluated in three drought stress and one non-stress experiments in the field. QTLs important in rice adaptation to drought have been saturated and refined with additional markers. For example, consistent QTL, RM8085-RM3825 on chromosome 1 for grain yield under drought in TPE, with additive effect from the landrace has been narrowed down to 42.8 Kb (from 1.6 Mb) between RM11873-RM3825. Another QTL for yield under drought was mapped at RM11943 on chromosome 1 with additive effect from IR20 and is linked to *sd1* locus governing plant height. Similarly, a consistent QTL for days to 50% flowering (DFF), RM314-RM6836 on chromosome 6 was also narrowed down to 279.8 Kb (from 4.9 Mb) between RM19715-RM19727. This QTL harbours *Hd1* locus regulating heading date. A QTL for yield under drought was also mapped on chromosome 6 near RM314. Both these QTLs for DFF and yield are located within the meta-QTL for yield under drought (MQTL6.1). These consistent QTLs for yield and DFF under drought in TPE with linkage to *sd1* and *Hd1* loci mapped using rice lines derived from locally adapted landrace may be useful in MAB of rice cultivars suitable for water-limited environments.

b) A total of 28 genomic regions associated with for phenology and plant production traits were mapped using a subset of 132 IR62266/Norungan recombinant inbred lines under nonstress and drought in TPE. Consistent QTLs were detected for phenology and production traits on chromosomes 1, 2, 3, 6 and 10. The QTLs for days to flowering, biomass, spikelet fertility and grain yield under drought identified on chromosome 1 (RM5389-RM11943), chromosome 2 (RM324-RM6374-RM7426 and RM12460), chromosome 3 (RM16030 and RM3387-RM3392) and chromosome 6 (RM585-RN217) in this study have been earlier reported as meta-QTLs for yield under drought viz., qDTY1.1, qDTY2.1 and qDTY2.2, qDTY3.1 and qDTY3.2 and qDTY6.1, respectively. These regions were also found to be QTL hot spots for various secondary traits contributing in drought resistance in rice. Thus, these consistent QTLs for yield and secondary traits could be ideal candidates in MAB for

drought resistance in rice. Further, QTL on chromosome 6 was syntenic to QTLs for yield and secondary traits under drought in major cereals. Map based cloning of genes underlying the QTL will be useful in functional genomics analysis and genetic engineering of drought tolerance in rice and other cereals.

ii. Genome wide association mapping of plant production and root traits under drought in TPE using diverse rice lines

A total of 49 diverse rice accessions, including traditional landraces native to the target production environment, were evaluated for phenology, plant production and root traits under natural drought stress in rainfed target populations of environment (TPE) in six successive field trials from 2010 to 2015. Significant variation for phenology, plant production and root traits under drought was noticed among the accessions. Genotyping of the rice accessions using 599 polymorphic simple sequence repeat (SSR) markers showed considerable genetic variation among them. STRUCTURE analysis grouped the 49 accessions into three subpopulations. Similarly, three clusters were observed in neighbor joining tree created using Nei's genetic distance. The sub population, POP1 consisted mostly of landraces, while subpopulation POP3 consisted of advanced breeding lines and POP2 with accessions from all groups. Genome-wide association mapping detected 61 markers consistently associated in two or more trials with phenology, plant production and root traits under drought in TPE. The markers, PSM52 (Chr 3), RM6909 (Chr 4), RM242 (Chr 9) and RM444 (Chr 9) were consistently associated with grain yield and root traits. The markers, PSM127 (Chr 3) and PSM133 (Chr 4) were consistently associated with yield, plant height and spikelet fertility. These markers with pleiotropic and consistent associations with yield and secondary traits under drought in TPE may be robust candidates for MAB for drought resistance in rice.

iii. Evaluation of root QTL introgression lines for root growth and plant production under drought in TPE

Roots are considered to contribute in drought resistance in rice. Genetic improvement of root system through conventional breeding is hampered due to difficulty in phenotyping root traits. Marker assisted breeding (MAB) will hasten development of genotypes with improved root system. Consistent QTLs for root traits have been mapped leading to MAB for improved root traits in rice. However, a positive impact of improved root traits on grain yield under drought is yet to be demonstrated, especially in target populations of environment (TPE). Precise phenotyping of root system is critical for understanding the biological value of roots in drought resilience. Thus rice lines introgressed with root QTLs were evaluated in the field for root system and plant production under managed stress and drought in TPE as well as under managed stress. Rice lines showed considerable variation in root growth and yield under stress in these different systems of evaluation. The agronomic value of the root QTLs in terms of yield under drought, especially in TPE will be discussed.

Six Decades of Arecanut Breeding

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The present paper reflects the history, research and development and also introduction, conservation, evaluation of germplasm and breeding strategies that led to the release of high yielding varieties for the past sixty years. Research on various aspects of arecanut had been initiated way back in 1956 with the establishment of Central Arecanut Research Station which later merged in 1970 to form ICAR-Central Plantation Crops Research Institute, a premier research institute under ICAR, with coconut, arecanut and cocoa as its mandate crops, with its headquarters at Kasaragod, Kerala. The Institute maintains a total of 176 arecanut accessions in the field gene bank located at its Regional Station, Vittal (Karnataka), known to be the largest assemblage in the world. These include 153 indigenous and 23 exotic accessions collected from different arecanut growing tracts within the country and other parts of the world.

For the past more than sixty years, explorations were undertaken to collect the diversity in the crop and also conservation and cataloguing of germplasm. A total of 75 accessions have been described based on morphological, reproductive and fruit traits and an Arecanut Germplasm Database has been created and uploaded in the ICAR-CPCRI website. Systematic evaluation of germplasm through selection has led to the release of nine varieties, *viz*., Mangala, Sumangala, Sreemangala, Mohitnagar, Swarnamangala, Madhuramangala, Nalbari, Kahikuchi and Shatamangala for commercial cultivation in different agro-climatic zones of India. Evaluation of germplasm resulted in identification of donor parents for desirable traits which are being exploited in improvement of the crop.

To overcome the difficulties in climbing the tall palms, dwarf germplasm has been exploited to breed dwarf hybrids with high yield potential, by crossing Hirehalli Dwarf (HD) and released tall high yielding varieties. Two hybrids *viz*., HD × Sumangala and HD × Mohitnagar were found to be promising and released for commercial cultivation as 'VTLAH-1' and 'VTLAH-2'.

A protocol has also been standardized for mass multiplication of elite planting materials of dwarf hybrids and YLD tolerant palms. Tissue cultured plants are under evaluation for disease resistance/tolerance at different experimental plots in YLD endemic areas.

Future breeding activities will encompass arecanut genome sequencing for facilitating marker assisted selection and molecular breeding, trait-specific germplasm collection, evolving dwarf varieties with greater yield potential, abiotic/ biotic stress tolerant varieties and also development of varietal specific biochemical/ molecular markers.

Development and Use of Mapping Populations in Crops: Genetic Considerations

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The development of molecular marker technology has caused renewed interest in genetic mapping. An appropriate mapping population, suitable marker system and the software for analyses of data are the key requirements for a molecular mapping and molecular breeding programme. Genetic map construction requires that the researchers: (i) select the most appropriate mapping population(s); (ii) calculate pair wise recombination frequencies using these population; (iii) establish linkage groups and estimate map distances; and (iv) determine map order.

A population used for gene mapping is commonly called a mapping population. Mapping populations are usually obtained from controlled crosses. Decisions on selection of parents and mating design for development of mapping population and the type of markers used depend upon the objectives of experiments, availability of markers and the molecular map. Different types of mapping populations that are often used in linkage mapping are: (i) F2 population; (ii) F2 derived F3 (F2:F3) populations; (iii) Backcross Inbred Lines (BILs); (iv) Doubled haploids (DHs); (v) Recombinant Inbred Lines (RILs); (vi) Near-isogenic Lines (NILs) and (vii) Chromosomal Segment Substitution Lines (CSSLs). Besides the above-mentioned populations, Bulk Segregant Analysis approach, using any one of the above-mentioned populations (except NILs) is frequently used in gene tagging.

The genetic segregation ratio at maker locus is jointly determined by the nature of marker (dominant / codominant) and types of mapping populations. Therefore, a thorough understanding of the nature of markers and mapping population is crucial for any mapping projects. Further, precise molecular and phenotypic characterization of mapping population is vital for success of any mapping project. Since large mapping populations are often characterized by different marker systems, map construction has been computerized. Computer software packages, such as Linkage1, GMendel, Mapmaker, Mapmanager and Joinmap have been developed to aid in the analysis of genetic data for map construction.

Trends in Oil Palm Breeding

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Oil palm **(***Elaeis guineensis* Jacq**.)**, the highest yielding crop in the world has been introduced into our country to meet the edible oil demand**.** This has the potential of yielding annually 4-6 tonnes of oil/ha, which is several times more than that of other oil yielding crops like groundnut, sesame, mustard, sunflower, coconut etc**.** It is generally agreed that the Oil palm **(***E. guineensis***)** originated in the tropical rain forest region of West Africa**.** The main belt runs through the southern latitudes of Cameroon, Cote d'Ivoire, Ghana, Liberia, Nigeria, Sierra Leone, Togo and into the equatorial region of Angola and the Congo. Colombia is the main oil palm producer in Latin America. In order to increase the narrow genetic base of the existing oil palm cultivars, collection activities are carried out for *Elaeis guineensis* in African counties including Angola and Cameroon. In India oil palm was introduced as an ornamental crop in 1860 where four palms were planted at National Botanical Garden, Calcutta. The first commercial planting of oil palm was taken up by the Kerala Agriculture Department at Thodupuzha in 1960 with *Dura* and *Tenera* germplasm imported from Malaysia and Nigeria. In oil palm significant immediate improvement in commercial planting material has been realized by changing over from crosses within restricted population such as *Deli Dura,* to crosses between *Dura* and *Pisifera* from different unrelated populations. The present day oil palm planting materials were derived from four oil palm trees of unknown origin planted at the botanical garden in Bogor, Indonesia in 1848. At present the *Deli Duras* derived from Bogor, forms the bulk of mother palms in commercial seed production in Malaysia and Indonesia. In India, major research work on oil palm improvement was initiated during 1974. The *Dura* population consisting of 300 palms available at Thodupuzha was the base to start the initial selection programme in which 40 superior palms were selected.

Like other cross-pollinated species, the major objective in breeding oil palm is heterosis breeding (development of hybrids) and population improvement. With the advent of concept of single gene inheritance of shell thickness, breeding programmes have largely been focused on phenotypic mass selection combined with family selection in bi-parental crosses for production of *Dura,* which in combination with *Pisifera* would give high yielding *Tenera*.

The oil palm breeding programme in past have predominantly been directed towards high oil yield. In recent years focus on other traits like superior oil quality, dwarfness, compact canopy, long stalk, big kernel *etc.* have also got attention of breeders.

- High oil yield and bunch analysis
- Superior oil quality
- Development of location specific hybrids
- Long bunch stalk
- Screening for drought tolerance/ high water use efficiency
- Screening for disease resistance/ tolerance
- Compact canopy and slow growth

In 1920s, discovery of inheritance of shell thickness at Yangambi station in Zaire, led to commercial plantations of *Tenera* hybrids. Open pollinated material from one *Tenera* palm (the famous djongo) from Eala and nine from Yawenda formed the Yangambi type population. The breeding materials at the NIFOR, Nigeria were derived largely of *Duras* from Calabar and *Tenera* from Aba and Ufuma (Hartley, 1988). The Institute de Recherches pour Les Huiles et Oleagineux (IRHO) breeding materials is derived from 38 palms selected from Pobe in Benin and four palms selected at Biongerville in Ivory Coast. The latter provided the well known population; the L2T *Tenera* and its selfed progenies have produced some outstanding *Pisifera*. A major systematic evaluation of various oil palm breeding stocks was tested by IRHO in an "International Experiment" in 1946 and resulted in the adoption of reciprocal recurrent selection as the main breeding procedure in oil palm improvement programme. A number of research institutes have been set up to spearhead the crop improvement research on oil palm. In India major research work on oil palm improvement was initiated during 1974. The *Dura* population consisting of 300 palms available at Thodupuzha was the base, fromwhich 40 superior palms were selected. Also, *Tenera* hybrids were produced by crossing 11 such superior *Dura* palms with *Pisifera* pollen grains imported from Nigeria. These hybrids were evaluated at the then Central Plantation Crops Research Institute (CPCRI), Research Centre at Palode during 1976 and two hybrids were selected as the promising ones with a potential of yielding 4.6 tonnes of palm oil per hectare under rainfed conditions, designated as Palode-I and Palode-II.

Although the traditional plant breeding based on phenotypic selection is very effective, it has suffered from several limitations for complex traits. Unlike morphological and biochemical markers, DNA markers are basically unlimited in number and are not affected by environmental factors and/or the developmental stage of the plant. The molecular markers were transformed from the earlier RFLP markers to the highly variable and effective SNP markers. The most widely used markers in the recent times are SSR and SNP markers for several purposes like genetic diversity, linkage maps, and for GWAS studies. Genetic diversity and relationship of American and African germplasm have been studied using AFLP and RFLP markers. Recently, genetic relationship between elite oil palms from Nigeria and Malaysia have been studied using SSR markers. Likewise different workers used different molecular markers on different oil palm germplasm for various purposes of genetic diversity studies. Genome wide SNP discovery and identification of QTLs associated with agronomic traits in oil palm using genotyping-by-sequencing (GBS) has resulted in development of a linkage map, spanning 1429.6 cM, with an average of one marker every 1.26 cM. Most of the agro-morphological characters are controlled by multiple Quantitative Trait Loci (i.e. complex traits). Genetic mapping of these functional loci facilitates marker assisted breeding. Two of the most commonly used tools for dissecting complex traits are linkage analysis and association mapping.. The association mapping offers several advantages, of which few like, it requires natural population, high resolution of mapping the QTLs, more allele number, less time and broader reference population are important. However, till now in oil palm very few reports on association mapping have been published. In addition to the use in breeding for specific traits through MAS, the molecular markers are also useful is diagnosis and characterization of diseases, determining legitimacy of genotypes/ progenies, protection of IPR *etc*. Oil palm in Southeast Asia is badly affected by basal stem rot (BSR) disease caused by *Ganoderma boninense*. Breeding for resistance is an obvious approach and a long-term solution for Ganoderma disease. Construction of linkage maps may be continued until a fully saturated map is developed and is accessible for common use. There is a need for joint ventures, involving beneficiary countries and reputed laboratories, for this purpose.

Trends in Coconut Breeding

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Coconut, *Cocos nucifera* L., a monoecious perennial palm, is a monotypic species under the genus *Cocos* and placed in the family Arecaceae (formerly Palmaceae). It is an important oil yielding palm, cultivated in the coastal humid tropics. The origins of the coconut palm are the subject of controversy, with two divergent points of thought: most authorities claiming South Asian origin, while others claim its origin in West Coast of Central America and in the adjacent islands of the Pacific. Recent studies, based on SSR profiles of 1,322 coconut accessions, representing the geographical and phenotypic diversity of the species, indicates independent origins of coconut cultivation in Pacific and Indo-Atlantic oceanic basins, with two highly differentiated corresponding subpopulations. Regardless of its origin, coconut has spread across much of the tropics, aided by sea currents and also sea-faring people.

The palm is referred to as 'Kalpavriksha', since it provides nutritious food and refreshing drink, oil for edible and non-edible uses, fibre of commercial value, shell for fuel and industrial uses, timber and a variety of miscellaneous products for domestic and industrial use. The coconut palm, though a monotypic species, exhibits variability in forms with several distinct populations and ecotypes, widely differing from each other in morphological characters. Coconut palms are commonly categorized into two groups, viz. Talls and Dwarfs. The Talls are the most commonly cultivated for commercial production in all coconut growing regions, while the Dwarfs are increasingly being grown for their aesthetic value as well as for consumption as tender nuts. Rising production costs and dwindling returns, requires efforts to increase productivity, which necessitates development of improved varieties suitable for different agro-ecological zones and for variable uses.

In contrast to annuals, breeding in plantation crops is extremely challenging and a long drawn process because of the long pre-bearing age, heterozygosity, mixed pollination, long time taken to attain optimum production, requirement of huge area for experimental planting, resources required for experimentation, low seed multiplication rate and lack of a clonal propagation technique. Despite these limitations, India was the first country in the world to take up systematic breeding programme and exploit hybrid vigour in coconut.

Collection, conservation and cataloguing of germplasm are accorded the topmost priority in coconut research. In India, germplasm collection was initiated in 1924 with introduction from the major coconut producing nations of South East Asia. Traditionally, conservation of coconut genetic resources has been undertaken in *ex situ* field gene banks. However, this requires large area, considerable manpower and financial resources for establishment and management of the gene bank. In recent years, considering the long economic life span of >50 years, *in situ* conservation is being advocated to help conserve a much larger genetic pool. Further complementary conservation of genetic resources through cryo-conservation of pollen and embryos is underway.

Characterization of coconut genetic resources has resulted in the identification of trait specific variability with reference to morphological traits, floral traits and pollen recovery, fruit component traits and tender nut water quantity and quality. Variability for fatty acid composition of coconut oil has also been observed among accessions. The quantity and quality of the coconut inflorescence sap has also been found to vary across accessions. Studies on the physio-chemical and structural characteristics of coconut fibres, with a focus on coconut coir industry, indicated varieties suitable for manufacture of stronger yarns, dyeing and production of α-cellulose. Screening on the basis of physiological and biochemical parameters has resulted in the identification of drought tolerant accessions. Breeding for disease resistance, especially root (wilt) disease has resulted in development of disease tolerant varieties. Further, accessions having low incidence of eriophyid mite damage have been identified.

Molecular characterization of coconut germplasm has been undertaken using a range of marker systems viz. RAPD, AFLP, DAF, ISTR, IISR and SSR markers. Characterization based on leaf polyphenols, isozyme analysis and protein polymorphism has also been attempted. Advances in genomics research have resulted in the development of markers based on single nucleotide polymorphisms as well as novel gene-targeted markers such as SCoT markers for characterization of germplasm/identification of candidate genes for marker assisted selection.

Selection and hybridization have been the two major techniques used in varietal development. Evaluation of promising accessions have resulted in the development and release of 29 improved varieties through application of mass selection. India was one of the first countries in the world to report on heterosis/hybrid vigour in coconut. Presently, more than 150 cross combinations have been developed for evaluation. Initially, DxT and TxD hybrid combinations were produced and evaluated. Subsequently TxT hybrids were evaluated and more recently DxD hybrids are under evaluation. So far 20 hybrids, have been released for cultivation in the country.

Work on tissue culture has been carried out with explants such as zygotic embryos, leaf base, apical meristem, endosperm, roots, leaf tissues and inflorescence. Experiments, to enhance production of embryogenic calli and regeneration of somatic embryos from embryogenic calli from plumular explants are in progress.

Work on development of mapping populations and genetic maps have also been attempted. With the advent of next generation sequencing technologies, transcriptome sequencing has turned out to be the technique of choice for large scale gene discovery. At CPCRI, transcriptome profiling of susceptible and healthy CGD palms have been undertaken, providing new insights into the interaction of coconut palms with root (wilt) disease pathogen and aid mapping of disease-resistant candidate genes. Work on coconut genome sequencing has been undertaken in CGD and the draft genome is 1.83 Gb with 51953 predicted genes. Unraveling of biochemical pathways, mainly oil and GA biosynthesis pathways, have been attempted to better understand the crop and facilitate marker assisted breeding.

Taking into consideration the challenges to coconut productivity as a result of climate change and other production constraints, more focused research and developmental initiatives are required for sustaining productivity levels of coconut plantations. In addition to developing varieties suitable for emerging market demands with focus on product diversification, there is a need for better understanding of the variability in the crop, development of trait specific donor lines, variety specific markers as well as trait specific markers to facilitate marker assisted selection to hasten the breeding programme.

Recent Trends in Cashew Breeding

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The current paper discusses the recent trends in cashew breeding at the ICAR-Directorate of Cashew Research, the nodal agency for cashew research in the country. The directorate harbors 545 germplasm accessions which are maintained in National Cashew Field Gene Bank. Further, in AICRP centers also, about 1104 accessions are conserved. Out of these 545 accessions, 502 accessions have been characterized and five catalogues have been published comprising of 478 accessions. Further, a core collection consisting of 61 accessions derived through a relatively new technique i.e. advanced maximization strategy with heuristic approach has been established in the field. An interactive decision support system for cashew germplasm management has also been developed to select the germplasm accessions based on multiple character combinations.

As cashew is highly cross pollinated, heterozygous and perennial in nature, crop improvement efforts are slow to yield results. Simple selection or hybridization followed by selection of superior genotypes and their propagation through vegetative means are the easiest breeding methods. Most of the released varieties (43) are either bred through selection or hybridization followed by selection. However, keeping in view of the current needs, the recent approaches in cashew breeding have been to, a) Develop dwarf and compact hybrids with high yield and better nut characters for high density planting through direct cross and back cross breeding, b) Development of bold nut types with high yield through hybridization, c) Generate variability for insect pest resistance/ tolerance i.e. Tea Mosquito Bug (TMB) and Cashew Shoot and Root Borer (CSRB) through wide hybridization program involving wild species like *A. microcarpum* with popular cultivars of cultivated cashew, d) Seedling selection in popular varieties, e) Mutation breeding for TMB resistance/ tolerance, f) Evaluation of cashew genotypes specifically for cashew apple types, g) Assessment of Cashew Nut Shell Liquid (CNSL) content and identification of high and low CNSL types, g) Identification of molecular markers linked to economic traits through SSR markers for selection in early stages of the plant.

The future strategies would include development of pre-breeding lines, varieties resistant/ tolerant to cashew stem and root borer, rigorous screening of germplasm for biotic and abiotic stresses, exploring the possibilities of apomixis and polyploidy breeding, development of new SSR markers and genetic engineering aspects.

Genetic Improvement of Coffee - Global Scenario and Current Trends in Breeding with Special Reference to India

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Coffee, the stimulating beverage crop is an important plantation crop cultivated in India. Popularly referred as `Brown Gold`, coffee is of great economic importance for more than 50 countries that grow coffee on commercial scale and earn substantial revenue from exports of coffee beans. Globally, coffee is the second largest traded commodity, next to petroleum products with an annual turnover of about US$ 70 billion. The global coffee production touched 159.6 million bags (60 kg) during 2017. In India, coffee is cultivated in an area of 4.4 lakh ha with an annual production of around 3.12 lakh tonnes. Coffee cultivation in India is a small holder enterprise with 99% of coffee farmers possess holdings of less than 10 ha and depends primarily on coffee for their livelihood.

Coffee belongs to the genus *Coffea* of family Rubiaceae and comprises of over 100 species. Commercial coffee production relies on only two species, *Coffea arabica* known as 'Arabica' and *C. canephora* referred as 'Robusta'. Arabica is reported to have originated in the high lands of Abyssinia in South West Ethiopia while Robusta coffee is native to Central Africa. In the genus *Coffea*, *C. arabica* L. is the only self-fertile, allo-tetraploid species and all other species including *C. canephora* are diploids and generally self-incompatible. In general, *C. arabica* is characterized by low genetic diversity, attributed to the allo-tetraploid origin, reproductive biology and evolution process of this species while the diploid species represent considerable variability and some of these species form valuable genetic resource for breeding. Further, the arabica and robusta coffee types differ in plant morphology, vegetative vigour, ploidy level, breeding behaviour, genetic diversity, yield potential, bean and beverage quality.

Arabica produces superior quality beverage but manifest susceptibility to several diseases and pests like leaf rust (*Hemileia vastatrix Berk & Br*), coffee berry disease (*Colletotrichum kahawae* Waller et Bridge), stem borer (*Xylotrechus quadripes* Chevrolat) and nematodes (*Meloidogyne* sp. & *Pratylenchus* sp.). In contrast, robusta is more tolerant to these diseases and pests but the bean and liquor quality are inferior to arabica. Therefore, the main objectives of genetic improvement in arabica have been focussed to improve production coupled with resistance while in robusta, production and quality improvement has been the major thrust.

Conventional breeding in coffee is quite often constrained by several limitations such as long generation cycles, ploidy variation, incompatibility and reproductive barriers. In spite of these constraints, significant achievements have been made in genetic improvement of coffee through systematically pursued breeding programmes especially in arabica, world over. Thanks to the coordination between the coffee breeding groups across the continents which helped in exchange of coffee genetic resources and exploitation of new genetic diversity by application of advanced selection and breeding methods. Generation of basic information on coffee genetics in Brazil and

also the establishment of Coffee Rust Research Centre (CIFC) in Oeiras, Portugal to work exclusively on coffee leaf rust pathogen (*Hemileia vastatrix*) were instrumental for progress of rust resistance breeding programmes in many countries especially India. Identification of several dwarf mutants of Arabica *viz.*, `Caturra`, `San Ramon` and `Villasarchi` with compact bush stature and high yield potential as well as spontaneous hybrids of robusta and arabica such as Hibrido de Timor (HDT) and Devamachy with high vigour coupled with disease resistance, facilitated the development of several high yielding and disease tolerant cultivars with compact bush stature that have been extensively cultivated across the growing countries.

In India, since the establishment of Mysore Coffee Experimental Station in 1925, primary focus has been on breeding for rust resistance coupled with high production, improved quality and wide adaptability. In line with the scope and objectives of breeding, CCRI has successfully utilized the available variability both in Arabica and Robusta and developed 13 improved arabica and three robusta genotypes for commercial cultivation by employing proven breeding methods of which introgressive breeding has been the most successful strategy.

Recent advances in DNA marker technologies and coffee genomics provide new opportunities to overcome some of the limitations of conventional breeding. In coffee, molecular marker techniques have been used for genetic diversity analysis of germplasm, introgression analysis, development of linkage maps, identification of QTLs controlling several agronomically important traits. Further, markers linked to specific genes that impart tolerance/ resistance for coffee leaf rust (CLR); coffee berry disease and root knot nematode have been successfully used for marker assisted selection (MAS). SCAR markers for rust resistant gene S_H3 introgressed from *C. liberica*, facilitated the pyramiding of S_H3 gene with genes of robusta (S_H6, S_H7, S_H8, S_H9) by MAS. With the advent of affordable sequencing technologies, there has been a shift in focus towards whole genome sequencing and the total genome sequence of *Coffea canephora* has been published with new insights into caffeine biosynthesis. The impact of climate change is clearly visualized in several coffee growing countries resulting in flare-up of diseases and pests as well as drought and posing new challenges for coffee cultivation. The presentation highlights the global trends in coffee improvement with special reference to India and current focus of coffee improvement towards climate resilience.

Past, Present and Future Trends in Cocoa (*Theobroma cacao* L.) Breeding

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Cocoa (*Theobroma cacao* L.), the chocolate tree, called as 'Food of Gods', native to Amazon basin, a beverage crop, became an important industrial and plantation crop of India through its economic produce, the beans. It adapted well and integrated into palm based cropping systems by effectively utilizing the land, air, shade, light and root space in irrigated arecanut, coconut and oil palm gardens of South India. Cocoa research at ICAR-CPCRI, Regional Station, Vittal started in 1962 with few introductions, later intensified with safe collection of 505 accessions from its centre of origin/distribution and international gene banks, through intermediate quarantine centres, conservation in national gene bank, cataloguing through morphological, biochemical and molecular characterization and evaluated for their adaptability in the introduced environment through assessment of precocity in bearing, compatibility reaction, stability of yield over years, productivity and quality. Effective breeding criteria were followed in individual tree selections, among seedling progenies as well as in the clonal selections, which ultimately reduced the evaluation cycle. Self incompatible and cross compatible parents were subjected to hybridization and hybrid vigour was explored and utilized through five progeny trials, which aimed at high dry bean yield and drought tolerance. Through these systematic breeding programme, eight varieties were developed, which are high yielding, have industrial value, suitable for different agro climatic conditions and tolerant to both biotic and abiotic stress. For hybrid seed production, Bi/ Poly-clonal orchards were established with parents of known parentage and performance and soft wood grafting was standardized for quality planting material production and scion banks/ compact blocks of varieties were established for bud wood supply. ICAR-CPCRI Regional Station Vittal nursery, is a model cocoa nursery recognized by National Horticulture Board. Elite clones and varieties developed were taken to comparative yield trials under arecanut/ coconut/ oil palm gardens in different densities, varying shade levels as well as under multi location trials including traditional and nontraditional zones. Qualitative improvement bagged cocoa excellence award for Indian beans, which will further help fetch premium price for our farmers. Since cocoa genome is sequenced, molecular techniques and bioinformatics tools were used in assessing the genomic resources and Cocoa EST and Cocoa STRESS gene database were developed. Through Directorate of Cashewnut and Cocoa Development, Kochi, research results were taken to growers by establishment of demonstration gardens and encouraging participatory plant breeding. Growing demand in chocolate industry, standards of Indian produce, achievements of research and development, procurement and grinding facilities with co-operatives and private sectors are well recognized by the international cocoa communities, specifically in the Asia Pacific region, to address the common production problems. As working partner in the International Group for Genetic Improvement of Cocoa through Bioversity International, ICAR-CPCRI is actively participating in the collaborative programme, currently towards climate resilient cocoa improvement. The challenges of conserving and management of genetic resources of this perennial crop in the introduced environment with systematic long term breeding strategies on quantitative and qualitative improvement, supply of quality planting materials, usage of advanced genetic and genomic tools, climate change issues, threat of established and emerging pests and diseases are well addressed by CPCRI and resulted in significant contribution for the sustainability of cocoa and its crop promotion in India.

Trends in Breeding of Spices

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Spices are high value and low volume, export oriented commodities, which yield aromatic and pungent principles commonly used for flavouring and seasoning of food and beverages. Spices also constitute a major portion of medicinal plant wealth of India and are widely used in indigenous medicines. India is the centre of origin and diversity for major spices like black pepper and cardamom and possibly for ginger and turmeric. Many spices typically have long breeding cycles and development and introduction of improved cultivars by plant breeders may require many breeding cycles and dozens of years. Like other sectors of horticulture, the spices also face highly dynamic situations arising from such factors as decreasing labor availability, increasing environmental concerns, cost of energy, climate change and epidemics of new and invasive insects and diseases. The generally reactive nature of response to these factors translates to the release of new cultivars only after such pressures have accumulated significant impact on production. A general overview of spices genetic resources, pre-breeding, precision breeding, and genomics approaches and achievements is presented.

In addition to the activities of the ICAR-Indian Institute of Spices Research (IISR) in Kerala, collections of spices germplasm are also maintained at various research centers under the All India Coordinated Research Project on Spices. Presently, around 9200 germplasm accessions of major spices are being maintained in field gene banks.

The crop improvement research efforts so far resulted in release of 72 improved varieties of spices in the country. The conventional and/ or biotechnological methods have been employed in evolving the high yielding varieties of spices.

Various biotechnological approaches have great significance in conservation, utilization and increasing the production and productivity of spices. Molecular markers were used for crop profiling, finger printing, molecular taxonomy, identification of duplicates, hybrids, estimation of genetic fidelity of micropropagated and *in vitro* conserved plants in many spices. The NGS based transcriptome approaches have clearly demonstrated their advantages over previously developed methods and are becoming the new standard for transcriptomics studies in spices.

Areas of focus for the future breeding programmes

- Establishing global gene bank of spices
- Black pepper breeding for multiple resistance by convergent breeding
- Association mapping in black pepper
- Identification of genotype specific molecular markers in spices
- DNA bar coding for phylogenetic studies
- Isolation and characterization of resistant genes through transcriptome approach (*Phythpthora* - black pepper, *Ralstonia/ Pythium* - ginger)
- Soft rot resistant ginger and ginger (fresh) with long shelf life
- Ginger varieties suitable for secondary agriculture

- Hybrid turmeric, turmeric genotypes with better curcuminoid profile and high yield
- Low input responsive spices varieties (organic plant breeding)
- Hermaphrodite nutmeg

Plant genetic resources collection, conservation, cataloguing and evaluation of spices; precision breeding for higher yield, better quality and tolerance to diseases; pre-breeding to develop noble breeding lines as well as concentrated efforts to popularize the released varieties among all the stakeholders have yielded significant dividends in the arena of spices research. Both conventional and biotechnological approaches have been used in evolving the improved varieties. The agenda set forth, in tune with the needs and leads, for the coming years are destined to turn a new leaf in spices research in the days to come.

Trends in Rubber Breeding

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The genus *Hevea*, belonging to the spurge family Euphorbiaceae (which also includes *Manihot* and *Ricinus*), is comprised of about ten species of varied habits from small shrubs to tall trees of which *H. brasiliensis* (Para rubber tree) yields more latex than any other species in the plant kingdom. The Para rubber tree, native to Amazon forests of Brazil, has only recently being domesticated outside its natural range of distribution after Sir Henry Wickham first collected seeds of *Hevea* in 1876. Para rubber tree is diploid (2n = 36; genome size approx. 1.47 Gb), monoecious, entomophilic and predominantly outcrossing. The tree produces latex (in specialized laticifer cells in the bark tissue) which is cytoplasmic fluid chiefly comprised of the rubber bio-molecule (*cis*-1, 4-polyisoprene) with global strategic importance constituting raw material for several thousands of natural rubber-based products including tyres. Natural rubber elastomer has unmatched physical and chemical properties making it far superior than synthetic rubber.

Almost all breeding programme in *Hevea* is mainly focussed on maximizing latex productivity. However, the above polygenic, latex-oriented mono-trait selection strategy has, at least in few cases of present day popular clones, ensured indirect selection for undesirable economic traits like high susceptibility to diseases. Conventional crop improvement strategies have resulted in phenomenal improvement in rubber yield per unit area from about 250 kg/ha/yr from seedling plantations to 3500 kg/ha/yr from modern day clones with the potential yield sometimes surpassing more than 4000 kg/ha/yr.

Breeding in *Hevea* followed principles of cyclical 'generation-wise assortative mating" where superior clones from one generation is used as parents of next cycle of progenies from which successor clones are selected and utilized. In the initial stage, seedlings (full-sibs/ half-sibs/ polycross progenies) are evaluated in seedling nurseries (nursery evaluation trial) at 2-3 years of age through test-tapping following modified Hanmaker-Morris-Mann method. Based on growth and test-tap data top 10-20% of the selections are vegetatively propagated to fix their genetic potential and then forwarded to first phase of field evaluation namely, 'clonal nursery evaluation' following replicated breeding design in a closer spacing (2.5 m x 2.5 m). Depending upon growth and test-tap yield performance of clones in third to the fifth year of planting in comparison to popular checks, top 20% selections are passed on to large-scale evaluation trials (LST). In LSTs, the selections as well as check clones are planted in normal spacing (5 m x 5 m) and evaluated for growth and yield performance from 7^{th} year of planting for three years after which a pooled analysis is carried out on yield data and suitability of clones for further evaluation in on-farm trials (OFTs) is determined. Based on performance of clones in multilocational OFTs, clones are finally released for large-scale commercial planting. Currently, under participatory clone evaluation (PCE) programme, top 10% of selections from clonal nursery trials are simultaneously evaluated in LSTs and OFTs in order to reduce the period required for commercial release of clones.

Earlier breeding programme was time-consuming since it was comprised of small scale trial (SSTs) evaluation stage after the initial seedling nursery evaluation and on-farm trials were carried out only after LSTs. In the modified breeding programme, the SST stage is circumvented by a more rapid clonal nursery evaluation process and LSTs and OFTs are simultaneously carried out. Thus, from a very long breeding cycle of 40 years, the present day breeding programme has been considerably reduced to less than 25 years. Although many of the modern day clones including the RRII 400 series clones have been developed through long breeding cycle, the resultant clones showed consistent growth and yield performance justifying the robustness of *Hevea* breeding strategy followed in India and several other rubber growing countries. Nevertheless, constrained with limitations like asynchrous flowering, lesser fruit set success from selected parental combinations *etc*., conventional breeding is still time-consuming.

Almost many of the modern clones have been derived from the Wickham base (named after the original collector) and initially, only a handful of clones were used in the breeding programme. Thus, the original *Hevea* genetic base is not wider enough to represent the natural genetic variation as found in the natural range of distribution. Later, in 1981 and thereafter, expeditions were carried out by the International Rubber Research and Development Board, Malaysia, and others, which resulted in collection and distribution of thousands of *Hevea* germplasm accessions and other *Hevea* species to several rubber growing member countries. In order to strengthen the *Hevea* genetic base, several clones with yield potential and disease resistance were also exchanged among countries under international clone exchange programme.

In order to accelerate breeding in *Hevea*, molecular tools were explored which resulted in development of several early generation molecular markers ranging from RAPDs to simple sequence repeats (SSRs) markers. Later, several advance generation markers like Expressed Sequence Tags (ESTs) and SNPs (single nucleotide polymorphism) were developed. These markers helped in assessing genetic diversity and identification of genotypes of *Hevea* as well as population structure of important fungal pathogens of *Hevea*. Mapping of quantitative trait loci (QTL) is an area offering wide scope for assessing polygenic inheritance of yield and other adaptive traits in *Hevea* in order to assist in molecular breeding. Identification of robust QTLs for yield and other economic traits will help in early selection of desired genotypes and considerably reduce breeding cycle. Studies in these lines have resulted in identification of several linkage maps including a saturated linkage map of *H. brasiliensis* with QTLs for resistance to SALB (South American Leaf Blight) pathogen. Recently, *DArTseq* technique has been used for identification of QTLs for resistance to leaf diseases caused by *Corynespora* and *Phytophthora*.

Conventional hybridization has limitations because not all economic genes are naturally transferrable. Hence, with an effort to transfer specific genes of interest, genetic transformation in *Hevea* resulted in successful transfer of economically important genes related to yield and adaptive traits. Efforts are underway for gene-pyramiding using multiple gene cassettes comprised of genes for yield and adaptation to stress environs and transgenic *Hevea* with MnSOD genes has been successfully developed and are ready for field evaluation. However, environmental concerns have so far restricted the transgenic to laboratory, confined only to glass/ green-houses. Although large-scale field trials are required to establish the superior performance of transgenic *Hevea*, preliminary results from early evaluation glass-house trials is encouraging. Nevertheless, conventional genetic engineering techniques are mostly based on random gene transfer systems. The above issue can be addressed through utilization of *engineered nucleases*

which allow more precise and targeted gene engineering, termed '*genome editing*'. For *genome editing*, which is actively considered in many crops and few perennials, four types of engineered nuclease systems are available namely EMNs or LAGLIDADG homingendonucleases (LHEs), often termed meganucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), RNA-guided endonucleases (RGENs) and the clustered regularly-interspaced short palindromic repeat (CRISPR) system. Of these, CRISPR/ Cas9 (CRISPR-associated protein 9) system offers scope for more targeted and precise gene transformation in *Hevea*.

In order to shorten the long breeding cycle of *Hevea* and to evolve early selection parameters, transcriptomics, metabolomics, proteomics and whole genome sequencing are also being explored globally. There has been revived interest in *Hevea* genome sequencing and this field is now rapidly progressing with several draft genome maps already published with variable genome sizes ranging from 1.3 Gb to more than 2.00 Gb indicating the complexity of systems in such studies. Although many of these genome maps have limited access, at least one such recent works has allowed open access to nucleotide sequences related to various traits. Information from such genome maps offer tremendous scope for identifying genes related to yield performance as well as other important economic and adaptive traits including disease resistance although they all need validation using large populations under diverse environmental conditions before actually incorporated in to operational breeding.

Although several advanced molecular breeding techniques are available for genetic improvement of *Hevea*, judicious selection of appropriate technique is required before their actual operational use. Latex yield, which is the most important trait in *Hevea*, is polygenic and greatly influenced by environment with very large G x E interaction. This is compounded by the fact that yield in *Hevea* is also believed to be greatly influenced by root-stock and scion interaction, as indicated by very large intra-clonal variation, although no single study has delivered conclusive evidence. Hence, effective genetic transformation with specific genes for yield improvement is a challenging task. Transgenic *Hevea* need rigorous validation in large-scale field trials under diverse environs before they are released for commercial planting. It is also very much crucial to understand the basic genetic principles related to mechanism of inheritance of a particular trait and their accurate heritability estimates using adequate progeny populations *etc*. before any QTL based study is attempted. Similarly, identification of QTLs is also a challenging task particularly of adaptive traits like disease resistance which involve rapidly evolving pathogen populations as clearly established in the case of SALB-causing pathogen and leaf-infecting phytophthoras. Association mapping is a more robust alternative strategy for identifying markers related to economically important traits in *Hevea* as marker-trait associations are more effectively established and successfully demonstrated in many crops and trees. Hence, molecular breeding in *Hevea* can only be successful if the technologies implemented are robust with multi-trait marker systems which are thoroughly validated with wide range of target populations under diverse environs. Nevertheless, development of '*molecular marker integrated breeding*' strategy where conventional breeding is supplemented and accelerated with sufficiently field-validated, simple, robust and widely available molecular markers, is need of the hour in *Hevea*, as in the case of many economically important trees, particularly which involve long breeding cycles.

Strategies for Breeding of Fruit Crops in India

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Fruit crops breeding is very slow, cumbersome and difficult to achieve desired results due to the, out breeding, high heterozygosity, sterility, parthenocarpy, apomixis, polyembryony, poor fruit, excessive fruit drop and the long juvenile period. The breeding methods such as introduction, selection, clonal selection, OP selections, half-sib raising, sib-mating, hybridization, mutation and ploidy manipulation are commonly used to breed a variety based on the crops specific problems (trait specific breeding) and locations. With the advent of biotechnology, the MAS, diversity analysis through markers, confirmation of hybridity with markers genomics, proteomics, transgenics, cisgenics and genome editing are being used as a tool to accelerate the breeding programmes. Although the breeding methods are same for this group of crops, resulting offspring requires huge space for evaluation and there is a need to refine the selection procedure depending on the crop. This comes in the way of improvement as understanding of the genetics and inheritance pattern of the quantitative traits become extremely difficult. In many of the fruit crops like mango, guava, grapes, papaya, annona and jamun seedlings have become varieties due to the selection practiced by farmers. On farm conservation of indigenous types in several of the fruit crops would be useful in isolating genotypes resistant to biotic and abiotic stress. The interspecific hybridization and in some cases intergeneric hybridization needs to be adopted to harness the genes, which will be of great value to the improvement of fruit crops. Hence, the selection of parents in a breeding programme becomes difficult. Parents when they are selected based on the phenotype and used in the breeding programme, progeny performance or the manifestation of hybrid vigour becomes unpredictable. The progenies also are influenced to a great extent by the environment. However, one of the advantages of the heterozygosity is that in the F_1 generation itself segregation will be maximum and the desirable trait in the progenies can be fixed by vegetative propagation. The absence of molecular markers and pre-selection indices in most of the crops is hampering the breeding programme. Trait specific breeding was extremely difficult but with the development of several biotechnological tools it has become less difficult now.

The important keys for successful fruit breeding programmes are;

- ✓ Collection, conservation, characterization and utilization of wild species
- ✓ On farm evaluation, conservation and utilization
- ✓ Crossability studies in wild species
- ✓ Development of genomic resources like markers, transcriptomes and whole genome sequence data
- ✓ Identification of lines resistant to biotic and abiotic stress
- ✓ Genetically diverge varieties, nomenclature ambiguity and removal of synonyms
- ✓ Substantial progeny population for selection of useful recombinants
- ✓ Understanding the inheritance pattern of quantitative traits
- ✓ Development of pre-selection indices

- ✓ Cryopreservation methodology for taking up crossing of varieties wherein synchronized flowering is not there
- ✓ Use of biotechnological tools for trait specific breeding and shortening the breeding time

At ICAR-IIHR, the Division of Fruit crops is mainly working on the genetic improvement of fruit crops (mango, papaya, grapes, guava, pomegranate, custard apple, fig, jackfruit, pummelo and underutilized fruits) for improved productivity, quality and resistant to biotic and abiotic stresses by adopting the breeding techniques such as hybridization, mutation, backcross method, OP seedling selection, raising of half sibs, distant hybridization and haploid breeding. The research activities have resulted in the development and release of high yielding hybrids/ varieties in various fruit crops: mango (5), papaya (2), guava (4), grapes (8), custard apple (1), pomegranate (1) and lime (1).

Division of Biotechnology has developed microsatellite markers for mango, pomegranate, sapota, garcinia, banana species and has taken up diversity analysis and structure analysis. Work on association mapping in mango is underway. Attempts are also being made to identify QTLs in banana for *Fusarium* wilt and drought tolerant traits. There are some new initiatives in the fruit crops improvement, such as rootstock breeding in mango, grapes and guava, polyembryonic studies in mango, TILLING in papaya, tissue culture in papaya, ploidy manipulation in pomegranate, mildew resistant breeding in grapes, breeding dwarf sapota and jackfruit varieties for high density planting, sweet pummelo varieties and bioprospecting of jamun varieties etc.