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COCONUT

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1. History, Origin and Distribution

Martius (1850) and Cook (1901) considered the West Coast of Central America as the centre of origin of coconut. On the other hand, de Candolle (1886) considered coconut to be of Asiatic origin. There are a lot of controversies pertaining to the origin of coconut. Whichever the place of origin of coconut, it is presently disseminated throughout the tropics.Coconut fossils have been discovered in India, New Zealand, Australia and South America, representing different continents of ancient Gondwana, except Africa and Antartica. Since coconut has a thick husk, which allows it to float on water for long distances, it could have been disseminated through seawater ensuring wide spread. Apart from this, migration of people from one place to other even as early as 1000 BC could also have caused its dissemination to wider areas. During 16th century, European explorers had taken coconuts to West Africa, the Caribbean and America. Much of these assumptions are now supported by studies on the genetic structure of coconut populations as assayed through molecular markers. Gunn *et al.* (2011), utilizing data from molecular studies, have proposed independent origins of coconut cultivation in Pacific and Indian Ocean basins.

2. Botany

Coconut (*Cocos nucifera* L.) is a monocotyledonous palm belonging to the family Arecaceae. It has only a single species '*nucifera*' in the genus *Cocos*, with the chromosome number of 2n=32. The palms have a robust, cylindrical, erect stem with a single growing point from where the successive leaf production takes place producing a terminal crown. Palms can grow up to 20-30 meters in tall varieties and 10-15 meters in dwarf varieties. Leaves are pinnate and are called 'fronds', which are generally 4 to 6 m in length and 1.5 to 2 m in width. Leaves have a strong rachis to which the leaflets are attached on both sides. Around 400 leaflets

are present in a frond. Leaflets are linear- lanceolate. Canopy of coconut ('crown') consists of 28 to 36 fronds at the tip of the stem arranged in circular or semi circular shape. Generally, in an adult palm, one frond is added to the canopy every month and one frond is abscessed from the stem. The inflorescence of coconut emerges from the axil of each frond every month. Inflorescence is protandrous. Unopened inflorescence looks like a spadix within a spathe. It takes 44 months from inflorescence primordial initiation to nut maturity. In a crown, one can see all stages of inflorescence. In the 'spadix', the pistillate flowers and staminate flowers are attached to spike like rachillae. As many as 200 to 300 male flowers and only one or a few female flowers are attached to these rachillae. Male flowers are found 1 to 3 together, sessile and pale yellow in colour with three small sepals, three larger petals and six stamens in two whorls. They have a rudimentary pistil. Female flower is solitary, larger than male flowers in size, globose in bud, enveloped by two small scaly bracteoles, three sepals and three petals, ovoid at anthesis, sub-oricularm sub-equal, persistent and enlarging in fruit, pistil with large trilocular ovary, three sessile triangular stigmas and three nectaries near the ovary base. Within two to three weeks after the spadix opens, pollination takes place. Coconut is mainly a cross pollinated crop. But the 'dwarf' type coconuts are predominantly self-pollinated. It takes 12 months from pollination for a pistillate flower to develop in to mature nut. Fruit is a globose, ovoid or ellipsoidal fibrous drupe. Tender coconuts are generally 7 to 8 months old. Fruit (nut) has an outer greenish pericarp, fibrous middle mesocarp and hard endocarp (shell). Inside the endocarp, the fruit consists mainly of solid, white endosperm (kernel), liquid endosperm (nut water) and a single embryo. Coconut has an adventitious root system, which goes to the depth of 1.5 to 2 meters but with a horizontal spread of 4 to 5 meters. Decayed roots are replaced regularly due to the formation of new roots.

Generally the nut matures 12 months after fertilization. Mature nuts can germinate soon after harvest. The embryo enlarges and the apical part emerges from the shell. The cotyledon develops into haustorium, which supports the growth of seedlings by absorbing nutrients from solid endosperm and nut water. Shoot emerges out of husk in about 2 to 3 months after sowing. After another 1 to 1½ month's time, the first leaf starts unfolding. Leaves, which are initially formed, remain unsplit until seedling has 8 to 10 leaves. The subsequent leaves split to give a pinnate shape. Usually seedlings are field planted a year after germination. Coconut is propagated only through seed nuts.

Palms remain juvenile for the next three to four years and then the

first inflorescence emerges from the leaf axil. Generally dwarfs produce inflorescence early (3-4 years) as compared to talls, which take 5 to 7 years for flowering. Hybrids come to flowering in 3 to 5 years after germination. Dwarf palms have a productive life span of 40-50 years. On the other hand, Tall type palms can survive for 80-100 years and can give economically profitable yields for more than 60 years. Generally, yield during initial years after flowering is not stable and palms attain stable yield only 15 to 17 years after germination. Some palms also have the tendency to exhibit partial alternate bearing phenomenon, where yields will be very low in one year and very high the following year (Menon and Pandalai, 1958).

3. Species and cultivars

The coconut palm, *Cocos nucifera* L., belongs to the monotypic genus with no known wild or domesticated relatives. However, the present day population of this palm presents a wide range of variability with several distinct populations and ecotypes, widely differing from each other in the morphological characters, particularly in respect of fruit characters and plant habit. A number of workers have attempted a classification of the various forms of coconut. A widely accepted classification puts cultivars into two groups - Talls and Dwarfs, on the basis of a few important characters like stature, growth characteristics of the palm and precocious nature of flowering, and nut and copra characters. In addition, certain variants have also been observed. One is the seedless coconut or male coconut tree, which produces only male flowers and another is spikeless coconut palm or spicata, wherein the inflorescence does not carry spikelets (the male and female flowers are borne directly on the primary spike). In coconut germplasm more than 300 types of cultivars exist. Apart from these, there exist a lot of natural variations, which are present in one or a few palms.

4. Genetics and Breeding

Coconut is a perennial crop, which takes at least two years to produce the F1 generation seedlings and 7 to 8 years for the F2 generation seedlings. Each female flower in an inflorescence has to be pollinated to obtain hybrid nuts, and an inflorescence can yield none to 20 nuts. Thus obtaining a large population of seedlings is also difficult. The problem is compounded by the fact that nut-setting percentage is very low particularly in hybridized inflorescences. Further, low germination percentage of coconut seed nuts, warrants undertaking of hybridization in a larger number of inflorescences to get the desired F1 population.

4.1. Cytology

Palms are difficult objects for cytological studies and hence have received little cytological attention in spite of their economic importance and biological and ecological significance. Santos was the first to study the cytology of *C. nucifera* in detail, and reported that the chromosome number of coconut as n = 16. In India, the chromosome number (n = 16) was reported in several publications (Janaki Ammal, 1945; Venkatasubban, 1945; Ninan *et al.*, 1960; Abraham *et al.*, 1961). These studies and those of several others (Nambiar & Swaminathan, 1960; Swaminathan & Nambiar, 1961; Raveendranath & Ninan, 1973) have confirmed the somatic chromosome number of 2n = 32.

4.1.1. ***Karyomorphology:*** A comparison of the gross features of chromosome complements of Tall (WCT) and Dwarf varieties reveals certain interesting facts. Raveendranath and Ninan (1973) observed that secondary constrictions were present on the long arm of chromosome VI in Talls and long arm of chromosome III in Dwarfs. However, these differences were not consistent and additional satellites were observed on chromosome II (long arm), chromosome I (short arm), short arm of chromosome XII (Raveendranath & Ninan, 1973), long arm of chromosome XII (Thankamma Pillai *et al.*, 1983) and IX (Nambiar & Swaminathan, 1960) in Talls and in chromosome VI (long arm) in Dwarfs (Raveendranath & Ninan, 1973). Nambiar and Swaminathan (1960) observed that in Talls, majority of the chromosomes had submedian centromeres, with two pairs of chromosomes much longer and three pairs relatively short. On the other hand, Raveendranath and Ninan (1973) observed that Talls as well as Dwarfs had a preponderance of chromosomes with median centromeres, with four submedian chromosomes (II, IV, VII, XIV) in WCT, three (chromosome II, VII, XII) in Dwarf Orange (DO) and only one (chromosome II) in Dwarf Green (DG). In higher plants, karyotypic evolution has been from complete symmetry to asymmetry (Stebbins, 1950). From this angle, WCTs show a more evolved karyotype than DO and DG. Total chromatin content is found to be greater in DG than WCT (Raveendranath & Ninan, 1973). The total chromatin content is more in wild species than cultivated ones. Therefore, DG appears to be the most primitive among the three cultivars studied. However, evidences from morphology, breeding system and meiotic behaviour support the possible evolution of Dwarfs from Talls.

4.1.2. ***Meiotic Studies:*** The different varieties of Talls and Dwarfs open pollinated and inbred populations show significant differences in their

meiotic behaviour. The Dwarfs are reported to show less stable meiosis than Talls, and it has been proposed that ancestral types show more stable meiosis (Lindquist, 1960). In general, microsporogenesis is more regular in open pollinated than inbred progenies. Nambiar et al. (1970) studied cytological behaviour of Laccadive Ordinary (LCT), Philippines Ordinary (PHOT), Andaman Ordinary (ADOT), New Guinea (NGT) and Cochin China (CCNT) varieties of coconut and observed that microsporogenesis was relatively regular in both inbred and open pollinated progenies of Laccadive variety, while comparatively higher frequencies of chromosome aberration and pollen sterility was observed in inbred as well as open pollinated progenies of Cochin China Tall and New Guinea Tall and inbred progenies of Philippines and Andaman varieties. The lack of inbreeding depression only in Laccadive variety could either be due to differences in intensity of inbreeding and selection between these geographically distinct varieties, or due to the Laccadive genotype being comparatively less sensitive to inbreeding.

Nambiar and Swaminathan (1960) observed many meiotic irregularities in Apricot from Straits Settlements and Dwarf Red forms, which are derived from the Dwarfs, while meiosis was regular in Laccadive Ordinary. Consequently, higher pollen sterility occurred in these two Dwarf derivatives in comparison to Laccadive Ordinary. Thankamma Pillai *et al.* (1983) studied meiosis in nine cultivars and hybrids and indicated that the percentage of abnormalities was highest in DG and DO, while chromosome abnormalities and sterility were very low in D x T and T x D hybrids. They concluded that the higher degree of inbreeding in Dwarfs might be the reason for higher chromosome aberrations and sterility in them. Cytological studies on *Spicata* variety (Ninan *et al.*, 1960; Ninan & Satyabalan, 1963) showed that meiosis was irregular with inversions, translocations and many other abnormalities. *Spicata* palms, being predominant outbreeders, are believed to have arisen from Talls through mutation. Further, cytological studies have been undertaken on abnormal palms, bulbiferous palms and root wilt affected palms.

Nambiar and Prasannakumari (1964) studied the effect of root (wilt) disease on microsporogenesis in coconut and observed low frequency of cytological aberrations, high pollen fertility and seed set. Thankamma Pillai and Vijayakumar (1972) studied the course of microsporogenesis in the progeny of a self-pollinated New Guinea palm, which produced defective nuts and observed aberrant meiosis. The sterility in this palm was attributed to inbreeding. Raveendranath *et al.* (1975) found no appreciable karyological differences between the Talls and abnormal

coconut palms producing bulbils in the place of inflorescences and opined that cryptic structural changes or genetic mutations might be responsible for the appearance of this type of coconut palm.

4.1.3. ***Cytology of endosperm and embryo:*** Abraham and Thomas (1962) reported free nuclear divisions in coconut water (liquid endosperm). But, this was disputed by Mondal *et al.* (1970), based on the biochemical analysis of coconut water. Abraham and Mathew (1963) and Abraham *et al.* (1965), observed that size of nuclei varied considerably in the developing endosperm, based on their studies on six month old nuts. They found that the tissues adjacent to endothelium were normally triploid (3x = 48), less frequently hexaploid (6x = 96) and still less frequently dodecaploid (12x = 192) and proposed that higher ploidy levels arise by C-mitosis. They also recorded an inverse relationship between ploidy and percentage oil content, with the inner part of the endosperm having the highest ploidy level and lowest oil content (Abraham, 1963; Abraham *et al.*, 1965). In the Tall variety, the percentage oil content in the outer, middle and inner layers of endosperm was 75.7, 54.1 and 41.4, respectively. Abraham *et al.* (1965) recorded higher ploidy levels (48x and above) in buttery endosperm (Philippines Macapuno coconuts), which they felt arose through amitosis and nuclear fusion. Unlike the endosperm, the young coconut embryos are diploids and divide by normal mitosis. Raveendranath and Ninan (1973) studied karyomorphological features of somatic chromosomes from six-month-old embryos and observed an essential uniformity in relative chromosome length from root tip (Nambiar & Swaminathan, 1960) and embryo cells of WCT palms. Ninan and Raveendranath (1965) reported occurrence of a haploid embryo in a WCT palm.

4.2. Crop Improvement

India ranks among the top three coconut producing countries in the world, with an annual production of 21665 million nuts. The coconut palm is cultivated across 18 states and 3 Union Territories in the country, in an area of 2.07 million hectares with a per hectare productivity of 10122 nuts/ha (Coconut Development Board, 2014). This average annual productivity is 68 nuts/palm contrasts sharply with the yield of 110 nuts/palm/year realized by a progressive farmer, 175 nuts/palm/year for T x D hybrids at research stations (Swaminathan, 1983) and 471 nuts/palm/year recorded in certain elite palms (Iyer *et al.*, 1979), indicating the vast scope for coconut improvement. Considering the fact that coconut belongs to a monotypic genus, with no known wild/domesticated relatives, the possibilities of tapping gene pools of related species is practically

nonexistent. Moreover, the available variability within coconut is being slowly depleted through large scale replanting programmes, thereby necessitating immediate collection and conservation of existing native populations.

The first organized coconut breeding in India was started in 1916 at the erstwhile Coconut Research Stations at Kasaragod and Nileshwar, now under the Central Plantation Crops Research Institute (ICAR-CPCRI) and Kerala Agricultural University, respectively. Central Plantation Crops Research Institute (ICAR-CPCRI) under Indian Council of Agricultural Research has the mandate to conduct research on production, protection and processing technologies of coconut. This is supported by the research programmes under the All India Coordinated Research Project on Palms implemented in 15 Centres located in nine State Agricultural Universities, one Central Agricultural University and two research institutes. Coconut production in the country has increased from 12678 million nuts (6952 nuts/ha) during the year 2001 to 21665 million nuts (10122 nuts/ha) in 2014 with about 3.5 percent compound growth rate of production, as a result of developmental initiatives and implementation of technologies generated from research institutes within the country.

ICAR-CPCRI has undertaken extensive explorations for collection of coconut genetic resources and now maintains the world's largest collection of coconut germplasm. Crop improvement research, encompassing enrichment of coconut genetic resources, characterization, utilization and evaluation of germplasm/hybrids has resulted in development of improved coconut varieties. Based on multi-location trials, superior lines have been selected and released for cultivation in different parts of the country. Till date, about 48 improved varieties of coconut, including 20 high yielding hybrids have been released for cultivation in India, with yield potential of 84-167 nuts/palm/year or 1.8-4.6 tonnes of copra/ha/year. Drought tolerant and disease resistant accessions have been identified and are being used to develop high yielding varieties/hybrids with tolerance to biotic/abiotic stress. Biotechnological research is in progress for clonal propagation, gene transfer and use of molecular markers for fingerprinting germplasm.

4.2.1. *Germplasm collection and conservation:* In India, germplasm collection began in 1924 with the introduction of cultivars from Fiji, Indonesia, Malaysia, Philippines, Sri Lanka and Vietnam (Cochin China) at the Central Coconut Research Station, Pilicode. Subsequently selfed and open pollinated progenies were planted at ICAR-CPCRI (then CCRS), Kasaragod, in the 1940s. The germplasm collection was intensified in 1952

and in 1958 the first indigenous germplasm survey and collection was started. In 1981, survey and collection was made from six Pacific Ocean countries under an FAO/IBPGR funded expedition, which added 24 exotic collections. During 1997-2001, with the objective of strengthening the germplasm in the International Coconut Genebank for South Asia hosted by India, explorations were undertaken by ICAR-CPCRI, with ADB funding, from the Indian Ocean Islands of Mauritius, Madagascar, Seychelles, Maldives, Comoros and Reunion, and the South Asian country of Bangladesh, and a total of 42 accessions were collected. Further ICAR-CPCRI collected four coconut germplasm from Sri Lanka, in the year 2000, under the Indo-Sri Lanka bilateral agreement. Extensive prospection and collection of indigenous coconut genetic resources, from different coconut growing regions of the country, was undertaken under National Agricultural Technology Project (NATP) on Sustainable Management of plant diversity and about 127 indigenous germplasm collections were made (1999-2004). Further, with funding from the Indian Council of Agricultural Research, distinct accessions have been conserved in the National Active Germplasm Site at the ICAR-CPCRI.

Presently, ICAR-CPCRI has the world's largest collection of coconut germplasm with 438 accessions from 28 countries, representing coconut germplasm of South and South East Asia, Caribbean Islands, Indian Ocean Islands, Pacific Ocean Islands and African countries, and India. The indigenous coconut germplasm, comprises collections from Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Goa, Gujarat, Orissa, West Bengal, Assam, Meghalaya, Bihar, Andaman and Nicobar Islands and Lakshadweep Islands. Twenty four Pacific Ocean collections and six Nicobar collections are available at the World Coconut Germplasm Centre (WCGC), Andamans. The WCGC was initially envisaged by ICAR-CPCRI as an off-shore quarantine centre and is now maintained as a germplasm conservation centre by ICAR-Central Island Agricultural Research Institute (formerly, Central Agricultural Research Institute), Port Blair. Some of the coconut accessions are duplicated and maintained at the centres under the All India Coordinated Research Project on Palms (AICRP on Palms), namely, Pilicode in Kerala, Aliyarnagar and Veppankulam in Tamil Nadu, Kahikuchi in Assam, Ambajipeta in Andhra Pradesh, Arsikere in Karnataka, Bhubaneshwar in Orissa, Jagadalpur in Madhya Pradesh, Sabour in Bihar, Mondouri in West Bengal, Navsari in Gujarat and Ratnagiri in Maharashtra, for evaluation and testing their regional adaptability. Germplasm characterization is undertaken using the IBPGR descriptor (Anonymous, 1991). ICAR-CPCRI has brought out descriptors for 74 coconut accessions (Ratnambal *et al.*, 1995, 2000) and a

coconut germplasm database has been developed at the institute. ICAR-CPCRI has also contributed to the development of the World Catalogue of Conserved Coconut Germplasm and Catalogue of farmers varieties brought out by COGENT/Bioversity International (Bourdeix *et al.*, 2010).

In addition to morphological descriptors, work on molecular characterization of conserved coconut germplasm is also undertaken at the institute. Characterization of coconut germplasm based on isozyme analysis (Parthasarathy *et al.*, 2004) and protein polymorphism (Geethalakshmi *et al.*, 2000) has been attempted. Chempakam and Ratnambal (1993) studied the variation in leaf polyphenols in coconut germplasm. Molecular characterization of the coconut germplasm in the country has been undertaken using a range of marker systems *viz.* RAPD, AFLP, DAF, ISTR, IISR and SSR markers and are described in the subsequent pages, under the section on biotechnology. *In addi*tion to characterization of germplasm, it is envisaged to develop a list of trait specific donor parents for desirable traits utilized in the coconut improvement programme both at the institute, various coordinating centres under the All India Coordinated Project on Palms (AICRPP) and State Agricultural Universities.

4.2.2. *Varieties released through selection:* Screening of the available coconut cultivars for their performance under different ecological conditions is a promising method of obtaining ecotypes suited for the different regions. In India, selection and evaluation of promising coconut accessions conserved both at the institute as well as the various coordinating centres under the All India Coordinated Research Project on Palms as well as State Agricultural Universities have resulted in the development and release of 28 improved varieties (Niral *et al.*, 2014).

At ICAR-CPCRI, on the basis of a preliminary evaluation of available coconut cultivars, promising cultivars were selected for further multi-location trials. Laccadive Ordinary Tall was found to be a superior yielder in the states of Andhra Pradesh, Kerala, Tamil Nadu and Maharashtra and was therefore released by ICAR-CPCRI in 1985 under the name 'Chandra Kalpa' (Anon., 1985). Based on the evaluation of five local Banawali types of coconut (Banawali Green Round, Banawali Green Long, Banawali Yellow Round, Banawali Yellow Long and Banawali Red Round) in the Konkan region of Maharashtra, Banawali Green Round which recorded the highest average yield of 142 nuts per palm per year was selected and released during 1987 by the Konkan Krishi Vidyapeeth, Dapoli, for cultivation in the Konkan coast under the name 'Pratap' (Anon, 1987; Nagwekar *et al.*, 2003). A selection from Andaman Ordinary Tall, with

greater tolerance to drought, high copra (176 g) and oil content (70%) and higher annual nut yield of 92 nuts/palm was released as VPM-3 by CRS Veppankulam, TNAU during 1994. A selection from Philippines Ordinary Tall, an exotic cultivar, has been recommended for release as a National Variety (Kera Chandra) by the XII Workshop on AICRPP (December, 1995) for commercial cultivation in the West Coast, including Konkan regions, coastal Andhra Pradesh and West Bengal (Anon., 1996). During 1999, in the XIV AICRPP workshop, Assam Agricultural University released a high yielding selection of Assam Green Tall for cultivation in Assam under the name Kamrupa. Kerala Agricultural University (KAU) during 2006 released Kera Sagara, a high yielding selection from the exotic accession Seychelles Tall with high copra content of 203 g, for cultivation in the state of Kerala. ICAR-CPCRI during 2007, based on an evaluation of 16 coconut accessions planted in 1972 in RBD, identified high yielding selections from Cochin China Tall, Java Tall and Andaman Giant Tall. Subsequently these improved selections were recommended for release in the XVIII AICRPP Workshop, for cultivation in different regions of the country as Kalpa Pratibha, Kalpa Mitra and Kalpa Dhenu, respectively, and released and notified by the Central Sub-committee on Crop Standards, Notification and Release of variety vide Notification of Ministry of Agriculture (Department of Agriculture and Co-operation) S.O. 1714(E) dated July 18, 2008. A semi-tall selection of Malayan Green Dwarf with higher tolerance to root (wilt) disease and good tender nut quality was identified during 2007 and released and notified for cultivation as Kalparaksha by ICAR-CPCRI during 2008. These four varieties have been registered with PPV & FRA under the extant variety category (Anon, 2015). During the year 2007, the XVIII Biennial workshop of the All India Coordinated Research Project on Palms also recommended for release: Kalyani coconut 1, a selection from Jamaica Tall, from CRS Mondouri BCKV as a dual purpose copra and tender nut variety for cultivation in West Bengal; Gauthami Ganga, a high yielding selection from Gangabondam from CRS Ambajipeta, as dual purpose copra and tender nut variety for cultivation in Andhra Pradesh; Kera Keralam, a high yielding selection from WCT as a national variety for cultivation in the states of Tamil Nadu, Andhra Pradesh, Kerala, Maharashtra proposed by CRS, Aliyarnagar; Kera Bastar, a selection from Fiji Tall, proposed by CRS, Jagdalpur as a national variety for cultivation in the states of Chhattisgarh, Tamil Nadu, Andhra Pradesh, Maharashtra. Kera Bastar and Kera Keralam were subsequently relased and notified for cultivation by the Central Sub-committee on Crop Standards, Notification and Release of variety vide Notification of Ministry of Agriculture (Department of Agriculture and Co-operation) S.O. 1714(E) dated July 18, 2009. Chowghat

Green Dwarf, with higher resiatnce to coconut root (wilt) disease was identified by ICAR-CPCRI for release as a dual purpose copra and tender nut variety and was recommneded for release by the XIX Biennial workshop of the All India Coordinated Research Project on Palms during 2009 and subsequently notified for cultivation in the root (wilt) affected tracts of the country by the Central Sub-committee on Crop Standards, Notification and Release of variety (CVRC) vide Notification of Ministry of Agriculture (Department of Agriculture and Co-operation) S.O. 456(E) dated March 16, 2012. The XIX AICRPP workshop in the year 2009 also recommended the release of a high yielding ball copra variety Kalpatharu, a selection of Tiptur Tall, for cultivation in the states of Kerala, Karnataka, Tamil Nadu, which was released and notified by the Central Sub-committee on Crop Standards, Notification and Release of variety vide Notification of Ministry of Agriculture (Department of Agriculture and Farmers Welfare) S.O. 1775(E) dated August 20, 2015. Aliyarnagar 2 (ALR 2), a selection of Arasampaatii Tall, has been released for commercial cultivation in Tamil Nadu by CRS Aliyarnagar, during the year 2010. Considering the superiority of selections of MYD and MOD for nut yield, coupled with tender nut water quality, the proposal by ICAR-CPCRI their release as dwarf tender nut varieties under the name Kalpa Jyothi and Kalpa Surya was recommended by the XXI Biennial Workshop of the All India Coordinated Research Project on Palms during the year 2012 and was approved for notification as national varieties by the CVRC during 2014. During the same period, ICAR-CPCRI also proposed the release of Kalpa Haritha, a tall statured, high yielding selection from Kulasekharam Green Dwarf with lesser incidence of eriophyid mite infestation, for cultivation in the states of Kerala and Karnataka, which was approved for notification by the CVRC during 2014 (Anon., 2015). KAU released Kera Madhura, a high yielding semi-tall, dual purpose variety for copra and tender nut purpose during the year 2013. During the year 2013, ICAR-CIARI (formerly CARI) proposed for release four dwarf varieties viz., CARI-C1 (Annapurna), for its dwarfness coupled with high copra content of over 240 g; CARI-C2 (Surya), for its dwarfness coupled with round orange coloured fruits and higher nut yield of 104 nuts/palm/year and for ornamental purpose; CARI-C3 (Omkar) with pear shaped yellow coloured fruits for ornamental purpose and CARI-C4 (Chandan) with bright orange coloured pear shaped fruits for ornamental purpose which was recommended for release and cultivation in Andamans by the XXII Group meeting of AICRP on Palms (Anon., 2014).

The yield performance and important features of the released coconut varieties suitable for cultivation in the different states of the country are given in the following tables (Niral *et al.*, 2010).

Table 1. Improved varieties developed for cultivation in the country through selection

Variety	Important traits	Nut yield (ha^{-1} $year^{-1}$)@	Copra yield (t ha^{-1} $year^{-1}$)@	Recommended states	Agency responsible for release
Chandra Kalpa	Drought tolerant, high oil - 72%	17700	3.12	Kerala, Karnataka, Tamil Nadu, Andhra Pradesh, Maharashtra	CPCRI
Kalpa Mitra	High nut and oil yield, Drought tolerant	15222	3.68	Kerala & West Bengal	CPCRI
Kalpa Dhenu	High nut and oil yield, Drought tolerant	14160	3.41	Kerala, Tamil Nadu & Andaman &Nicobar Islands	CPCRI
Kalpatharu	Drought tolerant, ball copra, high yield	20709	3.64	Kerala, Karnataka, Tamil Nadu	AICRPP, UHS Bagalkot, Karnataka
Pratap	High yield	20826	3.60	Maharashtra	Dr. BSKKV, Maharashtra
Kamarupa	High yield	17877	2.90	Assam	AAU, Assam
Aliyarnagar Tall 1 - ALR (CN) 1	High yield	22302	3.50	Tamil Nadu	TNAU
Kera Bastar	High yield	19470	3.18	Chhattisgarh, Maharashtra, Tamil Nadu, Andhra Pradesh	AICRPP, IGAU, Chhattisgarh
Kera Keralam	High yield	26019	3.53	Tamil Nadu, West Bengal, Kerala	AICRPP
Aliyarnagar Tall 2 - ALR (CN) 2	High yield	21240	2.89	Tamil Nadu	TNAU

VPM-3	High yield, drought tolerant	14868	3.41	Tamil Nadu	TNAU
Kera Sagara	High yield	17523	3.64	Kerala,	KAU
Double Century	High yield	23140	4.6	Andhra Pradesh	APAU
Kera Chandra	High yield	19470	3.86	Kerala, Karnataka, Konkan region, West Bengal	CPCRI Andhra Pradesh,
Kalpa Pratibha	High nut, oil yield, tender nut,	16107	4.12	Kerala, Andhra Pradesh, Tamil Nadu, Maharashtra	CPCRI
	Drought tolerant				
Kalpa Haritha	Dual purpose for copra and	20886	3.70	Kerala, Karnataka	CPCRI
	tender nut				
Kalyani Coconut	High yield, West Bengal	14240	3.9	West Bengal	BCKV, West Bengal
Gautami Ganga	Dwarf, green fruits	13260	3.60	Andhra Pradesh	ANGRAU, Andhra Pradesh
Kera Madhura	Semi-tall, Dual purpose for	24480	4.80	Kerala	KAU, Kerala
	copra and tender nut				
CARI-C1 (Annapurna)	High copra content, tender nut purpose, green colour fruit	20231	1.41	Andaman & Nicobar Islands	CARI, Andamans
Chowghat Orange	Dwarf, orange colour fruit; tender	12852	-	All coconut growing regions	CPCRI
	Dwarf				
	nut purpose				
Kalpa Jyothi	Dwarf, yellow colour fruit; tender	19935	-	Kerala, Karnataka, Assam	CPCRI
	nut purpose				

Kalpa Surya	Dwarf, orange colour fruit; tender nut purpose	21593	-	Kerala, Karnataka, Tamil Nadu	CPCRI
Kalparaksha	Semi-tall, green fruits High nut, oil yield in RWD prevalent areas; suited for tender nut purpose	1326017748*	2.85	Kerala, Karnataka, Tamil Nadu	CPCRI
Kalpasree	Dwarf, superior oil, high yield in RWD areas	18360	1.8	CPCRI	CPCRI
CARI-C2 (Surya)	Ornamental purpose, orange colour fruit	24072	1.77	Andaman & Nicobar Islands	CARI, Andamans
CARI-C3 (Omkar)	Ornamental purpose, yellow colour fruit	16373	1.67	Andaman & Nicobar Islands	CARI, Andamans
CARI-C4 (Chandan)	Ornamental purpose, orange colour fruit	11505	1.80	Andaman & Nicobar Islands	CARI, Andamans

*- under disease free conditions in Kerala; @ - Yield estimated, at 7. 5m x7.5 m spacing, population of 177 palms ha^{-1}

4.2.3. *Hybridization:* Hybridization work in coconut was first started in Fiji (Marechal, 1928) but was discontinued due to economic crisis. In India, hybridization was initiated in 1932, followed some 15 years later by Sri Lanka. In the early 1960s, IRHO and its partners started their hybridization work, while other coconut growing countries started much later.

In India, hybridization programme was initiated with three intra-varietal and one inter-varietal cross at the Coconut Research Station, Nileshwar, in the year 1932, and was the first to report hybrid vigour in coconut (Patel, 1937). Over the years, more than 150 hybrid combinations involving indigenous as well as exotic tall and dwarf parents *viz.* Chowghat Orange Dwarf, Gangabondam Dwarf, Chowghat Green Dwarf, Malayan Yellow Dwarf, Malayan Orange Dwarf and Malayan Green Dwarf have been evaluated over the years at the CCRS, ICAR-CPCRI and various centres under the All India Coordinated Research Project on Palms and so far 21 hybrids have been released for cultivation, the yield potential of which varies from 95 to 141 nuts/palm/year and 13.20 - 26.40 kg copra/palm/year (Niral *et al.*, 2006, 2014).

In India, in view of the high yield performance, the hybrids COD x WCT, LCT x COD and WCT x COD were recommended for release from ICAR-CPCRI, from 1984 onwards under the name Chandra Sankara, Chandra Laksha and Kera Sankara, respectively (Anon., 1984-85). Subsequently, two more dwarf x tall hybrids involving MYD as female parent *viz.*, Kalpa Samrudhi (MYD X WCT) and Kalpa Sreshta (MYD X TPT) were recommended for cultivation by ICAR–CPCRI, during the AICRP on Palms Group Meeting during 2010 and 2014 (Anon, 2014, 2015; Jerard *et al.*, 2015). Subsequently, Kalpa Samrudhi was released and notified by the Central Sub-committee on Crop Standards, Notification and Release of variety vide Notification of Ministry of Agriculture (Department of Agriculture and Farmers Welfare) S.O. 1775(E) dated August 20, 2015.

KAU evaluated and released, from 1987, onwards five hybrids Laksha Ganga (LCT x GBGD), Ananda Ganga (ADOT x GBGD), Kera Ganga (WCT x GBGD), Kera Sree (WCT x MYD) and Kera Sowbagya (WCT x SSAT). TNAU evaluated and released three hybrids *viz.*, VHC-1 (ECT x MGD), VHC-2 (ECT x MYD) and VHC-3 (ECT x MOD) during the years 1982, 1988 and 2000, respectively. More recently, during May 2015, a Tall x Tall coconut hybrid *viz.*, LCT x CCNT, which recorded the highest mean annual nut yield of 198 nuts/palm/year and cumulative nut yield of 135 nuts/palm at CRS Veppankulam, TNAU has been

recommended for cultivation in Tamil Nadu as VHC-4 during the XXIII Group Meeting of AICRP on Palms (Anon. 2015).

Andhra Pradesh Agricultural University during 1992 released a high yielding tall x dwarf hybrid Godhavari Ganga (ECT x GBGD) for cultivation in Andhra Pradesh. A significantly higher yielding T x D hybrid, ECT X GBGD, has been recommended for cultivation in the Konkan region by AICRP on Palms CRS Ratnagiri, MPKVV under the name Konkan Bhatye Coconut Hybrid 1, in the year 2007 during the the XVIII Biennial workshop of the All India Coordinated Research Project on Palms (Nagwekar *et al.*, 2012).

Much later during 2013, based on an evaluation of dwarf x tall hybrid combinations involving Gangabondam Dwarf as the female parent, the significantly high yielding hybrids *viz.*, Vasista Ganga (GBGD x PHOT) and Ananta Ganga (GBGD x LCT) proposed by CRS Ambajipeta, Dr YSR Horticultural University, Andhra Pradesh were recommended for cultivation in Andhra Pradesh and Karnataka during the XXI Group Meeting of the All India Coordinated Research Project on Palms. The hybrid GBGD x FJT, which showed significantly higher yield over the control at the AICRPP CRS Arsikere, UHS Bagalkot, was recommended for cultivation in Karnataka under the name Kalpa Ganga, by the XXI AICRPP Group Meeting (Anon., 2014).

The important features of the hybrids recommended for cultivation in the different regions of the country is given in Table 2).

4.2.4. *Breeding for specific traits:* Coconut breeding programme, in addition to yield improvement, are also aimed at development of drought tolerant and pest resistant varieties. Farias Neto *et al.* (2003) estimated the coefficient of repeatability for production of fruits and solid albumen in coconut palm using statistical methods of the variance analysis, main components (covariance and correlation) and structural analysis (covariance). Significant variability was detected between hybrids for fruits and solid albumen productions. Repeatability coefficients obtained by the variance analysis and structural analysis (correlation) showed the smallest values. If the level of 90% is considered sufficient to select hybrids with relative superiority for fruit and solid albumen productions based on the estimation of repeatability by the main components method (covariance), then it would be recommended to perform five and three evaluations for fruit and solid albumen productions, respectively. They reported a high degree of variability for copra yield, dehusked nut weight, nut yield, copra weight and whole nut weight and all these characters showed high

Table 2. Coconut hybrids released for commercial cultivation in India

Hybrid Variety	Source population of parents	Important traits	Nut yield@ (ha^{-1} $year^{-1}$)	Copra yield@ (t ha^{-1} $year^{-1}$)	Recommended states	Agency responsible for release
Chandra Sankara	COD x WCT	High yield	20532	4.27	Kerala, Karnataka, Tamil Nadu	CPCRI
Kera Sankara	WCT x COD	High yield, drought tolerant	19116	3.78	Kerala, Karnataka, Maharashtra, Andhra Pradesh	CPCRI
Chandra Laksha	LCT x COD	High yield, drought tolerant	19293	3.76	Kerala, Karnataka	CPCRI
Kalpa Samrudhi	MYD x WCT	Dual purpose variety, Drought tolerant, higher nutrient use efficiency	20744	4.35	Kerala, Assam	CPCRI
Kalpa Sankara	CGD x WCT	Tolerant to root (wilt) disease, high yield	14868	3.20	Root (wilt) disease prevalent tracts	CPCRI
Kalpa Sreshta	MYD x TPT	Dual purpose variety, High yield	29227	6.28	Kerala, Karnataka	CPCRI
Laksha Ganga	LCT x GBGD	High yield	19116	3.73	Kerala	KAU
Ananda Ganga	ADOT x GBGD	High yield	16815	3.63	Kerala	KAU
Kera Ganga	WCT x GBGD	High yield	17700	3.56	Kerala	KAU
Kera Sree	WCT x MYD	High yield	23364	5.05	Kerala	KAU

Kera Sowbhagya	WCT x SSAT	High yield	23010	4.49	Kerala	KAU
VHC-1	ECT x MGD	High yield	21240	2.87	Tamil Nadu	TNAU
VHC-2	ECT x MYD	High yield	25134	3.74	Tamil Nadu	TNAU
VHC-3	ECT x MOD	High yield	27612	4.47	Tamil Nadu	TNAU
Godavari Ganga Pradesh	ECT x GBGD	High yield	18585	2.79	Andhra Pradesh	ANGRAU, Andhra
Konkan Bhatye coconut hybrid 1	GBGD x ECT	High yield	20532	3.47	Maharashtra	Dr. BSKKV, Maharashtra
Kalpa Ganga	GBGD x FJT	High yield, suitable for ball copra production	21417	3.38	Karnataka	UHS, Bagalkot, Karnataka
Vasista Ganga	GBGD x PHOT	High yield	22125	3.88	Andhra Pradesh, Karnataka	Dr YSR Horticultural University (Dr. YSRHU), Andhra Pradesh
Ananta Ganga	GBGD x LCT	High yield	22656	3.84	Andhra Pradesh, Karnataka	Dr. YSRHU
VHC-4	LCT x CCNT	High yield	28497	4.27	Tamil Nadu	TNAU

(@ - Yield estimated, at 7. 5m x7.5 m spacing, population of 177 palms ha^{-1})

heritability and genetic advance. The copra yield in coconut was strongly and positively correlated with nut yield, copra weight, kernel weight, whole nut weight and dehusked nut weight. The direct effects of dehusked nut weight, percentage of husk to whole nut weight, percentage of kernel to whole nut weight, copra weight and nut yield on copra yield were positive and high. These characters are to be given emphasis while selection for improvement of copra yield in coconut (Ganesamurthy *et al.*, 2002a). Niral *et al.* (2009), based on studies of fruit component traits in the Indian germplasm collection conserved at CPCRI, which includes 58 tall accessions and 13 dwarf accessions, reported highest variability for endosperm content and fruit weight, and lesser variability for the traits viz., oil content and thickness of endosperm. Their studies indicated that oil yield/ha and copra yield/ha is more influenced by size of the nut and endosperm content and not by oil percentage or endosperm thickness *per* se. High copra content of >300g was recorded in the accessions, San Ramon Tall (SNRT), Malayan Tall (MLT) and Markham Valley Tall (MVT), while low copra content >100g was recorded in Surinam Brown Dwarf (SUBD), Pattukottai Green Dwarf (CGD 01), Chowghat Green Dwarf (CGD) and the tall accessions Laccadive Micro Tall (LMT) and Nu Fella Tall (NUFT). In general, low copra and oil content was recorded in the dwarf accessions. The correlation coefficients indicated highly significant positive correlation of fruit weight with weight of all fruit component traits, *viz.*, husk, shell, kernel, copra and oil content per fruit. The thickness of the husk did not show any significant correlation with any of the fruit component traits. On the other hand, the percentage of husk showed significant negative correlation with nut weight kernel weight and copra weight, indicating the need to select accessions with lesser husk percentage while breeding for higher endosperm/oil yield. Copra yield/ha and oil yield/ha showed highly significant positive correlation among themselves, indicating that these are not influenced by the fruit component traits alone. Therefore, the fruit weight, husk percentage and nut yield appear to be the most important characters influencing coconut oil output per hectare and hence it is possible to improve coconut oil productivity in the coconut plantations by selection of superior accessions based on these traits.

Copra is the major product of coconut in India, which is used for extraction of oil and edible purposes. Milling copra is used for extraction of oil, while edible grade of copra is used for consumption. Best quality edible copra is in the form of ball copra and about 45,000 t of ball copra is produced annually in the country. Traditionally, production of ball copra is limited to certain coconut tracts and all coconut varieties are not utilized

for ball copra production. Since ball copra fetches a premium price in trade, a preliminary study was undertaken to assess the suitability of 17 coconut accessions for ball copra production. Spoilage of copra due to germination of nuts during storage was observed and the percentage of spoilage varied across accessions. Recovery of ball copra was highest in Laccadive Micro Tall (LMT) followed by Tiptur Tall (TPT) West Coast Tall (WCT) and Java Tall (JVT), indicating the superiority of these tall accessions for ball copra production (Niral *et al.*, 2010). High yielding selections of WCT, JVT and TPT have been released as improved varieties *viz.* Kera Keralam, Kalpa Mitra and Kalpatharu, respectively.

4.2.4.1. *Drought tolerance and climate change studies:* Coconut palm requires an average monthly rainfall of 150 mm for ideal palm growth and good nut yield and unlike annuals, the adverse effect of drought persists for the subsequent two to three years. In India, coconut is cultivated mainly as a rain fed crop in peninsular India, which accounts for 90% of the coconut area in the country, and is exposed to the vagaries of monsoon, resulting in poor yields. Therefore, there is a need to evolve drought/moisture stress tolerant coconut varieties.

Rajagopal *et al.* (1991, 2007), Chempakam *et al.* (1993), Kasturi Bai *et al.* (1996), and Naresh Kumar *et al.* (2000) revealed the possibility of identifying drought tolerant cultivars based on different anatomical, physiological and biochemical parameters, viz., accumulation of epicuticular wax on the leaf surface, low stomatal frequency and leaf water potential, the activity of enzymes like glutamate oxaloacetate transaminase (GOT) and acid phosphatase. Rajagopal *et al.* (1988, 1990) screened different coconut genotypes for drought tolerance and found West Coast Tall (WCT), Federated Malay States (FMST), Java (JVT), Fiji (FJT), Andaman Giant (AGT), LCT x GBGD (Laksha Ganga) and LCT x COD (Chandra Laksha) to be drought tolerant. Rajagopal *et al.* (2007), undertook genetic analysis of drought responsive physiological characters in coconut using line x tester analysis, involving two dwarf lines (CGD,MYD) and four tall testers (ECT, PHOT, LCT, FMST). Their studies indicated differential response in the seedlings for drought sensitive traits viz., transpiration rates, lipid peroxidation, photosynthetic rates (*Pn*) and water potential. They reported both additive and non-additive gene actions in the expression of above traits. Higher SCA for transpiration rate was reported, indicating heterosis for this character. Photosynthetic rates were observed to be governed by non-additive gene action and can be exploited for yield improvement through heterosis breeding. They concluded that the nature of gene action governing some of these drought sensitive traits

can be exploited in selective breeding for drought tolerance.

The identified drought tolerant genotypes are currently being used in the breeding programs at ICAR-CPCRI, Kasaragod, to evolve high yielding, drought tolerant hybrids. A technology evaluation trial of the identified lines and hybrids has been established in drought prone Sivaganga district of Tamil Nadu during 2009 and evaluation is in progress.

Further, from the perspective of climate change scenarios of atmospheric greenhouse gases CO_2 levels are predicted to increase from current levels of 380 ppm to between 500 and 970 ppm by the end of the twenty-first century (IPCC, 2007). The predicted increase in greenhouse gas concentrations is likely to result in global temperature increase, predicted increase between 1 and 5.5 °C for 2100. Coconut, being a C3 crop, is likely to benefit due to an increase in CO2 as in case of other C3 species.

A simulation model Infocrop-coconut developed by Naresh Kumar *et al.* (2008) indicates that coconut productivity is likely to increase by up to 10% during 2020, up to 16%, in 2050 and up to 36% in 2080 over current yields only due to climate change. However, in the west coast of India yield is projected to decline by about 2% in 2020, 8% in 2050 and 31 % in 2080 scenario over current yields due to climate change. Yields are projected to go up in Kerala, Tamil Nadu, Karnataka, Maharashtra while they are projected to decline in the states of Andhra Pradesh, Orissa and Gujarat. Since, climate change is projected to raise temperatures and affect rainfall patterns, it is important to understand the impacts of high temperature and drought on coconut. Furthermore, the increased biomass production by changes in atmospheric CO2 concentrations is likely to impose higher plant nutrient demand, acquisition and utilization (Cavagnaro *et al.*, 2011).

Hebbar *et al.* (2013), studied the interaction effect of climate change variables elevated CO_2 and elevated temperature (ET) with drought and nutrients on growth and development of coconut seedlings in an open top chamber (OTC) and reported that coconut seedlings in elevated CO_2 treatments accumulated significantly higher biomass of 1.13 and 1.98 kg seedling-1 with 550 and 700 ppm CO_2 respectively as against 1.10 in ambient treatment. The stomatal conductance (gs) and transpiration (Tr) of plants grown under elevated CO_2 was reduced without affecting the photosynthesis, thereby the whole plant WUE of coconut seedlings grown under elevated CO_2 was high. These workers reported that WUE significantly reduced both in high temperature and drought stressed coconut

seedlings, unlike in other annual crops where WUE increases with water deficit stress (Hebbar *et al.*, 1994). They concluded that the COD coconut cultivar selected for this study has insensitive stomata and hence the photosynthesis per unit water transpired is low, and as a consequence the WUE significantly reduced in stressed seedlings. Elevated CO_2 to certain extent compensated for water stress and high temperature induced reduction in growth of coconut. However, these workers concluded that even with high CO_2 the yield under drought was low because most of the energy is wasted for non-photochemical quenching (qN).

4.2.4.2. ***Insect resistance:*** A number of insect pests attack coconut palms, of which, rhinoceros beetle and red palm weevil are the major ones. These respond to conventional plant protection measures and therefore no specific breeding programmes for developing resistant genotypes have been initiated. Preliminary screening of cultivars/ hybrids against leaf eating caterpillar, Nephantis serinopa Meyr. (Kapadia, 1981) and rhinoceros beetle, Oryctes rhinoceros Linn. (Nambiar, 1991) indicated variations in susceptibility among cultivars, though no resistant variety was observed.

Eriophyid mite (Aceria guerroronis Keifer) has become a major problem in most of the coconut growing regions of the country and has drastically reduced the nut yield as well as quality of nuts. As it is very difficult to completely eradicate the pest through conventional plant protection measures, the necessity of identifying eriophyid tolerant varieties assumes greater significance. Different workers have reported association of fruit characters with mite resistance: shape (Julia and Mariau, 1979; Mariau, 1986; Moore and Alexander, 1990), colour of the fruit (Moore and Alexander, 1990), tightness of tepals (Howard and Rodriguez, 1991), gap between the rim of the fruit (Aratchige *et al.*, 2007) and aestivation of tepals (Lawson-Balagbo *et al.*, 2007). Arunachalam *et al.*, (2013) undertook digital phenotyping of tender coconut fruits and morphological traits associated with eriophyid mite infestation and reported color of inner tepal and firmness of the three month old tepals as major traits associated with infestation by eriophyid mite. High degree of mite tolerance was reported in a Cambodian variety with tight tepals (Mariau, 1986). At CPCRI, very less mite infestation was reported in fruits of COD and Kulasekharam Green Dwarf (KGD), while LCT and SSAT showed severe mite infestation. Kalpa Haritha, a high yielding tall selection of KGD, released for commercial cultivation in the states of Kerala and Karnataka, recorded lesser incidence of eriophyid mite infestation (11.55 infested nuts) amidst heavy infestation (53.9% infested nuts in WCT) on other palms in the vicinity (Niral *et al.*, 2014).

4.2.4.3. *Disease resistance:* Coconut is affected by a number of diseases, of which the major ones are *Phytophthora* bud rot, stem bleeding, Thanjavur wilt/Ganoderma disease and root (wilt) disease. Among these, root (wilt) disease is the most serious and in the absence of effective control measures against the disease, evolving resistant cultivars is of utmost importance. Studies on identifying coconut genotypes resistant /tolerant to root (wilt) disease were initiated by Varghese in 1934. Since 1961, the ICAR-CPCRI Regional Research Station, Kayangulam, has made considerable efforts to screen the available cultivars for tolerance to root (wilt) disease. However, all the cultivars/ hybrids screened were found susceptible to the disease (Menon *et al.*, 1981). Only the cultivar CGD, has been found to have field tolerance of over 90% to the disease (Anon., 1972). A survey of the disease-affected areas ('*hot spots*') identified some high yielding, disease free WCT and CGD palms (Iyer *et al.*, 1979). Subsequently, disease-free COD palms were also identified in the root (wilt) endemic tracts of Kerala.

Since 1988 a comprehensive breeding programme for utilization of field tolerant WCT,CGD and COD palms is in progress at the ICAR-CPCRI Regional Research Station, Kayangulam. Phenotypically and serologically disease-free WCT and CGD palms are used in the breeding programme to produce different cross combinations - WCT x WCT, WCT x CGD, CGD x WCT, WCT x COD, COD x WCT, WCT self, CGD self and COD self. In addition, mixed pollen from all selected healthy palms in the diseased tract is also used for pollination to develop a gene pool of field tolerant palms. So far 2455 seedlings have been planted in the disease-affected areas for screening against root (wilt) disease (Anon., 1997). A few progenies of the cultivar 'Gudanjali' from Gujarat have also been planted for screening (Anon., 1994). Subsequently, healthy COD mother palms in the 'hot spots', have also been used in the resistance breeding programme (Anon., 1996). A few exotic cultivars screened against root (wilt) disease were found susceptible to the disease, though significant differences in disease intensity between cultivars were observed (Mathai *et al.*, 1985, 1991). Meanwhile, *inter se* and selfed nuts of the 24 exotic accessions from the South Pacific Ocean Islands are being evaluated at the WCGC, Andamans, and have been planted in the 'hot spot' areas for screening for resistance/tolerance to root (wilt) disease (Jacob & Rawther, 1991).

During the year 2005, a selection from Malayan Green Dwarf was identified by CPCRI as a promising RWD resistant variety with high yield suitable for large-scale cultivation in the root (wilt) prevalent areas. This observation was recorded from the Seed Production Farm of the Coconut

Development Board Farm at Neriamangalam, Ernakulam among the five dwarf varieties of coconut namely MGD, Malayan Yellow Dwarf (MYD), Malayan Orange Dwarf (MOD), Chowghat Green Dwarf (CGD), and Chowghat Orange Dwarf (COD). Kalparaksha, a semi tall selection of Malayan Green Dwarf with higher level of resistance to root (wilt) disease, higher nut yield and sweet tender nut water was developed and released for cultivation by CPCRI during 2007 (Nair. *et al.*, 2009).

From a screening trial of eight exotic and two indigenous coconut accessions established at CPCRI (RS) Kayangulam, it was found that Chowghat Green Dwarf (CGD) had the highest level of resistance to root (wilt) disease after six years of planting compared to other varieties (Nair *et al.*, 1999). Thus the studies from the natural survey as well as from the screening trials confirmed the higher level of resistance of CGD to root (wilt) disease (Nair *et al.*, 1996). Subsequently, a selection from CGD was released for cultivation in the root (wilt) tracts under the name Kalpasree in the year 2009.

At CPCRI RS Kayangulam, the CGD x WCT hybrid progenies planted in 1991 were found to be early bearing and tolerant to the disease. The relatively higher disease tolerance of these D x T hybrids, coupled with their high yield potential has resulted in the development and release of this hybrid, Kalpa Sankara for planting in the root (wilt) disease endemic areas during 2008 (Nair *et al.*, 1996, 2006; Regi *et al.*, 2013).

The other major diseases of coconut are bud rot caused by *Phytophthora pulmivora,* stem bleeding disease and Thanjavur wilt! Ganoderrna disease. As these can be controlled by conventional plant protection measures, at present no specific breeding programme has been initiated to develop a resistant genotype for these diseases. However, the available coconut germplasm is being evaluated to identify disease resistant types for utilization in the future breeding programme.

Devakumar *et al.* (2011) analyzed the population structure among the root (wilt) disease-resistant and susceptible WCT palms from 12 locations in the three disease-endemic districts of southern Kerala, using nine microsatellite markers. They reported identification of two-population structure among the resistant WCT mother palms and concluded that progenies resulting from the cross between these two diverse resistant populations are expected to have high heterozygosity and enhanced disease resistance. They suggested reorientation of the breeding strategies by making rational choice of parental combinations with divergent genotypes for ensuring better performance of the progenies.

4.2.4.4. ***Nut water quality:*** The consumption of tender nuts as a natural, nourishing and refreshing drink is becoming increasingly popular in our country. As a result of the high demand, tender nuts are being harvested from the existing Talls, sacrificing the quality of nut water and at the cost of valuable copra and oil. Therefore, at ICAR-CPCRI, Kasaragod, a study was initiated to identify a suitable cultivar for tender nut purpose during 1988. Among the 46 accessions screened through organoleptic tests and biochemical evaluation (Dhamodaran *et al.*, 1993), the accession Chowghat Orange Dwarf (COD), from the Chavakkad village in Thrissur District of Kerala, had the maximum total sugars (7 g/100 ml) and reducing sugars (4.7 g/100 ml) coupled with optimum sodium and potassium levels (Table 3). On the basis of the superior nut water quality, the X Workshop of the All India Coordinated Project on Palms (September, 1991), recommended the release of COD as a tender nut variety (Anon., 1991).

Table 3: Biochemical constituents of tender nut water and nut yield in 12 coconut cultivars (mean values for 1988-91)

Cultivar	Volume of water (ml)	Sugars (g/100 ml)		Free amino acids (mg/100 ml)	K (mg/l)	Na (mg/l)	Yield (nuts/ palm/ year)
		Total	Reducing				
New Guinea Tall	358	5.8	3.0	1.4	2258	21	73
Philippines Ordinary Tall	457	5.8	3.7	1.3	2273	24	113
Fiji Long Tongwan Tall	390	4.9	3.6	1.4	2641	29	105
SpikelessTall	275	5.3	3.2	1.7	2617	38	149
WCT	240	5.6	3.2	1.3	2797	37	92
Andaman Ordinary Tall	274	5.3	3.3	2.1	2272	27	94
Jamaican Sanblas Tall	263	6.0	3.4	1.7	2703	28	65
MYD	238	6.2	3.8	1.7	1998	36	53
MOD	303	6.7	4.1	1.8	2142	35	75
GBGD	267	5.6	3.5	1.7	2125	28	68
COD	351	7.0	4.7	1.8	2003	20	67
Guam I Tall	278	6.0	3.7	2.0	2434	34	96

Evaluation of tender nut traits in 74 conserved coconut germplasm at CPCRI Kasaragod, indicated wide variability in tender nut water content and quality (Ratnambal *et al.*, 1995, 2000). Based on evaluation of tender nut traits in various accessions and hybrids at CPCRI, both dwarf as well as tall varieties and also tall x dwarf and dwarf x tall hybrid varieties have been developed and released for cultivation in various agro-ecological zones. In addition, Dwarf x Dwarf hybrids have been developed and are under evaluation for their suitability for tender nut purpose. Improved varieties released for tender nut water quality attributes, are detailed in Table 4.

Table 4. Biochemical constituents of tender nut water in coconut varieties suitable for tender nut

Variety	Volume of water (ml)	TSS (°Brix)	Total sugars (g/100 ml)	Free amino acids (mg/100 ml)	Potassium (ppm)	Sodium (ppm)
Dwarf/Semi tall		-				
Chowghat Orange Dwarf	351	-	7.0	1.8	2003	20
Kalpa Jyothi	380	-	6.2	1.7	1998	36
Kalpa Surya	400	-	4.1	1.8	2142	35
Kalparaksha	290	-	4.92	-	2100	19.5
Kalpasree	240	-	4.8	-	2150	22.4
CARI-C1 (Annapurna)	470	5.4	2.52	1.48	2216	71
CARI-C2 (Surya)	154	6.3	2.27	1.52	2279	69
CARI-C3 (Omkar)	117	5.7	2.32	1.44	2133	62
CARI-C4 (Chandan)	198	4.9	2.38	1.44	2651	70
Tall						
Kera Chandra	450	-	5.86	1.3	2273	24
Kalpa Pratibha	448	-	5.5	1.1	2150	21.7
Kalpa Haritha	440	5.85	-	-	2100	17.5
Hybrid						
Chandra Sankara	347	6.58	5.99	1.73	2193	23.77
Chandra Laksha	339	6.51	5.72	1.76	2226	23.83
Kalpa Samrudhi	346	6.0	4.17	2.08	2370	35.1
Kalpa Sreshta	368	5.89	5.81	1.34	2081	33.3

Ganesamurthy *et al.* (2002 b) evaluated 40 tall and dwarf coconut genotypes for their physico-chemical properties during the tender nut age. The volume of coconut water was highest in 6-month-old tender nuts of tall genotypes and 7-month-old tender nuts of dwarf genotypes. The tall genotype San Ramon recorded the highest volume of coconut water (635.4 ml). The heaviest endosperm was observed in the tall genotype Andaman Giant Tall (207.7 g) and the dwarf genotype Andaman Orange Dwarf (140.4 g). The total sugar and mean reducing sugar content of coconut water increased with the ageing of nuts, with the dwarf genotypes recording higher total sugar and mean reducing sugar content than the tall genotypes. The mean potassium content decreased, whereas the mean sodium content and pH of the tender nut water of both tall and dwarf genotypes increased with the ageing of the nuts. The tall genotypes Zanzibar and West Coast, and the dwarf genotypes Chowghat Orange and Malayan Orange were superior in terms of tender nut water.

From the foregoing discussion, it is clear that research on genetics and breeding of coconut is going on for the past seven decades and the major research is being carried out in India. Considering the importance of the crop and the pioneering role played by India to preserve the coconut germplasm available in the coconut growing countries of the world, an International Coconut Gene Bank for South Asia was established in India under ICAR-Central Plantation Crops Research Institute. This would prevent coconut genetic resources from becoming extinct. Further, farmer participatory on-farm conservation is also being considered as an alternative conservation strategy to enable farmer participatory plant breeding in coconut. Focused characterization of germplasm for identification of more trait specific accessions is in progress and is being strengthened through molecular/genomic approaches to enable identification of genes/alleles for desirable traits and development of donor lines for effective utilization of the available diversity in coconut improvement. Identification of accessions with quality fibres is envisaged to further promote the coir industry as well as benefit the coconut farmer to a greater extent. Similarly, to promote value addition and enhance income generation of coconut farmers, specific genotypes suitable for production of coconut chips, higher recovery of inflorescence sap, and preparation of shell products need to be identified for utilization in the breeding programme. Dual purpose varieties for tender nut and copra production are being developed. t It is envisaged to develop superior genetic stocks, having greater homogeneity with the application of plumule culture technology, using selected mother palms of promising accessions, for utilization in crop improvement programme. Further,

intensive biotechnological research has to be carried out to address problems in clonal propagation, gene transfer and use of molecular markers for marker assisted selection in coconut.

5. Biotechnology

Research on coconut tissue culture was started in the eighties after success was reported in oil palm. It was initially thought that application of these techniques in coconut would result in success, this was proved wrong. The culture media developed for oil palm was indubitable for coconut and it was later proved that the coconut palm is highly recalcitrant to *in vitro* manipulations and every stage of the procedure brought its share of problems (Verdeil *et al.*, 1998). Besides, this technique would also help in the rapid propagation of elite hybrids. The success obtained in embryo culture and its use in germplasm collection has been one of the major achievements in this direction. The research on coconut tissue culture was thus aimed at solving the problems of phenol production using anti-oxidants other than activated charcoal, production of embryogenic calli and regeneration of plants. This research is of paramount importance because unlike in other crops, biotechnological research on coconut is being intensively carried out at present only at ICAR-CPCRI in India and a few laboratories abroad, although there have been sporadic attempts made in several other laboratories (Iyer, 1993; Iyer, 1995). Any breakthrough resulting in coconut biotechnology would be of great importance to the country, in general, and coconut growing states, in particular. Tissue culture of coconut has been carried out in several countries besides India including UK (Wye College), France (IRHO/CIRAD), USA (Florida University), the Philippines, Australia, Indonesia and Sri Lanka. As a result of these programmes, a few clonal plantlets have been produced over several years, but a repeatable and commercial protocol is yet to be achieved (Iyer and Parthasarathy, 2000; Parthasarathy and Bose, 2001).

A viable protocol for micropropagation of desired coconut hybrids/ selections is thus fundamental to disseminating the benefits of various breeding programmes among the farming community. The technique thus perfected could also be used for the mass multiplication of the disease resistant/tolerant types especially, in the context of the epidemic and devastating nature of root (wilt) disease in Kerala, which is estimated to cause a loss of more than 960 million nuts annually. Other international ramifications are the deadly diseases like lethal yellowing which is reported to be spreading at the rate of 100km/year in Mexico and would eventually wipe out all the country's estates (Veredeil *et al.*, 1998). Recently a lot of

interest has focussed on molecular aspects of coconut and markers including microsatellite and AFLP are being used to characterize the palms.

5.1 *Tissue culture:* Coconut is a difficult crop to manipulate *in vitro*. However, after Eeuwens' (1976) initial standardization of media and successful report of callus induction from various explant sources like stem, leaf, and inflorescence, a few laboratories around the world initiated intensive research. Till 1995, the work on coconut tissue culture was carried out sporadically in quite a few laboratories. Unlike many crops, coconut was posing many problems and besides this, the number of laboratories working on this crop was also less. Appreciating this, an international collaborative project was formed in 1995 consisting of researchers from France, Cote d'Ivoire, U.K., Germany, Philippines and Mexico. The results of this collaboration led to solving a large number of problems encountered in coconut tissue culture (Hocher *et al.*, 1998). Most commonly used basal medium at present is Y3 formulation (Eeuwens, 1976). However, del Rosario (1984) found no difference between Murashige & Skoog's (1962) and Y_3 media. Her work indicated that glucose was better for callus growth than sucrose. The major problem in coconut tissue culture has been the browning of tissue and its consequent death. To offset this problem, the antioxidant used is activated charcoal (AC), which adsorbs even auxins and kinetins such as 2,4-D and Benzyl Amino Purine to the tune of 99.4% and 97.8% respectively after five days of culture media preparation (Ebert *et al.*, 1993). This kind of inactivation of supplementa tion results in excess use of auxins and cytokinins. Oropeza and Taylor (1994) used radiolabelled 2,4-D to study the uptake by coconut inflorescence explants. The tissue took up most of the radioactivity within 24 hours. At this time the volume of the explant was only about one tenth of that of the external medium and the uptake of 2,4-D occurred against a concentration gradient. Thus, uptake of radio labeled 2,4-D by coconut inflorescence cannot be explained by simple diffusion. Alternatively, 2,4-D may be taken up by facilitated diffusion. They emphasized the importance of pH for 2,4-D uptake by coconut explants. Among other auxins used, one was 2,4,5-T, which led to the formation of nodular calli on inflorescence explants (Buffard- Morel *et al.*, 1988), and others like NAA and IAA resulted in direct embryogenesis in leaf explants (Raju *et al.*, 1984).

Plantlet development was first achieved at ICAR-CPCRI, Kasaragod from tender leaf tissue explants taken from 1-2 year old WCT seedlings (Raju *et al.*, 1984). However, it was not reproducible in subsequent trials. Profuse callus induction was achieved from immature zygotic embryos. Regeneration of somatic embryos from the embryogenic callus

has been achieved but plantlet differentiation is not regular. Several experiments in this direction are in progress. Somatic embryogenesis is usually indirect in coconut and has to pass through the callogenesis stage. Raju *et al.* (1984) reported direct embryogenesis and embryoid were reported to arise from vascular tissue but others reported this to be unusual as this area gives rise to root primordia normally. Karunaratne and Periyaperuma (1989) found that the embryogenic capacity of leaf explants was related to their physiological maturity in young palms of coconut. Leaf tissues from 12 to 24 month old palms were embryogenic but the potential was quickly lost with the onset of juvenility. Even in young palms, explants of tender leaves responded differently according to their maturity. Only a particular leaf in a particular state produces embryogenic cells and only a portion of this leaf yielded embryogenic explants (Karunaratne *et al.*, 1991). This may be one of the reasons why the experiments of Raju *et al.* (1984) were difficult to reproduce. Sporadic reports of success were reported using leaf explants by other workers also (Blake & Eeuwens, 1982; Shirke *et al.*, 1993; de Siqueira & Inoue, 1992; Verdeil *et al.*, 1994). Buffard Morel *et al.* (1992) reported successful production of somatic embryos from leaf explants. Their study was supported by detailed histological studies. According to them, the primary formations resulted from mitotic divisions of perivascular cells and differentiation of a cambium like layer insured the growth of nodular calli.

Tissue culture work with other explants such as zygotic embryos, leaf base, apical meristem and endosperm were also tried (Verdeil and Buffard Morel, 1995). Calli initiated from embryos, leaves, leaf bases, and apical meristem could not be regenerated (Neera Bhalla Sarin *et al.*, 1986). Calli induction from anthers and rachilla did not give repeatable response (Sarin and Suman Bagga, 1988). But root explants (Jones, 1983), and sub apical and leaf explants due to their limited embryogenic potential (Karunaratne *et al.*, 1991) have limited potential. Immature inflorescence and immature embryos have been found to be of promise. Blake and Eeuwens (1982) reported initial success using inflorescence tissue for callus production. They used immature rachillae on Y3 medium (Eeuwens, 1976) supplemented with 0.5µM NAA. Branton and Blake (1986) produced plantlets in 9 months from immature rachilla explants through somatic embryogenesis of nodular callus by reducing 2,4-D on Y3 medium to 100µM 2,4-D, with 5µM each of 2ip and BAP, and 0.25% AC. Areza *et al.* (1993) soaked the inflorescence tissue in antioxidants viz., citric acid (50mg/l) and ascorbic acid (100mg/l), prior to slicing and culturing in Y3 medium supplemented with activated charcoal, which resulted in less browning.

Verdeil *et al.* (1994) reported successful embryo maturation through somatic embryogenesis from inflorescence explants, which further regenerated into plantlets. They cultured immature inflorescences of coconut belonging to three different genotypes (PB-121, PB-111 & MYD), on an agar medium supplemented with activated charcoal (0.2%) and a range of 2,4-D (0.15 to 0.35 mM). Globular white callus emerged from immature floral meristems, depending on inflorescence age and 2,4-D levels. The use of immature inflorescences has been most successful among the different explants tried, and plantlet regeneration has been successful even though the transfer to nursery is yet to be achieved. The use of plumular tissues from germinating embryos has been another source from where success has been forthcoming, because of the juvenile nature of the tissue (Hornung, 1995). Bufford-Morel *et al.* (1995) used young non-chlorophyllous leaves and immature inflorescence in Eeuwens inorganic nutrients supplemented with Morel and Wetmore vitamins, 30 g/l sucrose, 2 g/l activated charcoal and 40 to 60 g/l 2,4-D. They observed calli after 6 - 8 months after culture initiation. They observed a multicellular pathway, which led to the formation of meristematic and epidermised structures with low 2,4-D (40 to 60 g/l). The first stage of development of these structures was characterized by the fragmentation of the cambium like zone and the formation of complex meristematic structures followed by their epidermisation. They observed a unicellular pathway, which led to the appearance and individualization of embryogenic cells isolated by a thick wall, with dense cytoplasm, a high nucleo-cytoplasmic ratio, and single large nucleolus and starch and protein reserves. This pathway was the result of the presence of high 2,4 - D concentration (80 - 120 g/l). Chan *et al.* (1998) developed a protocol using plumules of zygotic embryos. They used Y3 medium supplemented with 0.1mM of 2,4-D, 2.5 g/l activated charcoal, and solidified with 3g/l gelrite. The cultures were incubated for 3 months in darkness at 27°C. The calli bearing embryogenic structures were cultured in same medium with 1μM 2,4-D and 50μM BAP at a photoperiod of 12-hour light at 27°C, and subcultured every three months. Plantlets were produced after 6 to 9 months.

Rajesh *et al.* (2005) have outlined a procedure for regeneration of complete plantlets via organogenesis and embryogenesis from plumular tissues of West Coast Tall cultivar of coconut. Callus was induced from plumular tissues in Y3 media supplemented with either 2, 4-D (74.6 μM) alone or 2,4-D (74.6 μM) in combination with TDZ (4.54 μM). The frequency of callus induction increased and the browning of explants was reduced when a cytokinin (TDZ) was added along with the auxin (2,4-D) in the callus induction medium. The calli developed were subcultured at

monthly intervals to media containing lower levels of 2,4-D and a constant level of either cytokinins (BA and TDZ) or polyamines (spermine and putrescine). Higher percentages of embryogenic calli, somatic embryoids and meristemoids were obtained in Y3 media supplemented with either spermine or BA. Plantlets with balanced shoot and roots were transferred to pots and established in the greenhouse. Histological studies of the differentiated tissues confirmed the development of shoot buds (organogenesis) and typical bipolar embryoids (somatic embryogenesis).

Regeneration of complete plantlets via organogenesis and somatic embryogenesis was achieved from plumular tissues of two dwarf cultivars of coconut *viz.* Chowghat Green Dwarf (CGD) and Malayan Yellow Dwarf (MYD) (Rajesh *et al.*, 2014a). Significant differences were noticed between varieties for the formation of embryogenic calli. There were also significant differences for interaction between variety and regeneration medium with respect to formation of somatic embryos. Well developed plantlets were acclimatized under green house conditions and then successfully established in the field.

Long term maintenance of embryogenic calli is important as the totipotency is often lost in a short time *in vitro*. Bhavyashree *et al.* (2015) carried out a study utilizing 14 media combinations, with either 2,4-D or picloram as auxin source, for maintaining embryogenic callus obtained from plumular explants of coconut. Irrespective of type and concentration of auxins, callusing was observed in all the media combinations. However, high dose of 2, 4-D (above 74.6 µM) resulted in more browning and lesser percentage of callusing. Embryogenic nature of calli could be maintained to a maximum of 21 weeks in medium supplemented with 74.6 µM of 2, 4-D and subsequent culturing into higher concentration (90.4 µM). The embryogenic nature of maintained calli was tested by gene expression studies and it was revealed that genes such as *ECP, GST, LEAFY* and *WUS* were more highly expressed in long term embryogenic calli (21 week old) and genes such as *SERK, GLP, WRKY* and *PKL* in embryogenic calli (21 days old). The study concluded that coconut plumular calli could be maintained for longer periods without compromising on the embryogenic potential.

Griffis and Litz (1997) used anthers and filaments, unfertilized ovaries and immature leaf pieces. Both callus initiation and direct initiation of somatic proembryos were stimulated by addition of 2,4-D to the culture media. In a few cases, somatic embryos arose directly on filaments attached to immature anthers after several months in culture. Unfertilized ovaries

cultured in media supplemented with 2,4-D and diethylstilbestrol (DES) monitored for 24 months indicated substantial fresh weight gains and numerous unusual morphogenic changes in ovaries in Y3 medium supplemented with 5 or 15 mg/l DES, 25 or 50mg/l 2,4 -D and 3 mg/l 2iP. Several unfertilized ovaries formed callus and adventitious roots, but not somatic embryos. On similar media, some seedling immature leaf tissues formed callus on their cut edges while other formed roots or numerous somatic proembryos directly. Some proembryos also developed haustoria like tissues or roots and obvious bipolarity, but further shoot apical development did not appear except in one case.

Abscisic acid is also reported to induce somatic embryogenesis in coconut. Studies carried out by Adkins *et al.* (2002) have shown that whole immature, or mature sliced, zygotic embryos are a very good starting explant for coconut somatic embryogenesis. The highest rate of somatic embryogenesis was obtained when certain polyamines were added into the culture medium as well as activated charcoal (AC) to absorb unwanted phenolics. These past studies also showed that the development and maturation of the somatic embryos produced could be improved by the addition of abscisic acid (ABA), alone or with one of several osmotically active agents, into the culture medium.

Fernando and Gamage (2000) induced nodular callus from 7-9 months old immature zygotic embryos in BM72 medium supplemented with 24 µM 2,4-D. This callus was subcultured in to the medium supplemented with 2.5 - 7.5 µM abscisic acid for 3 - 7 weeks and subsequent subculture at 5 weekly intervals on media containing gradually reduced concentrations of 2,4 -D. They found incorporation of ABA enhanced the production of somatic embryos. These embryos formed normal plants. Studies by Samosir *et al.* (1999) have indicated that the development and maturation of coconut somatic embryos can be improved by using ABA alone or with any of the osmotically active agents, preferably PEG.

Immature zygotic embryo explants were more likely to undergo somatic embryogenesis than mature ones. Samosir *et al.* (1998) used longitudinally sliced mature zygotic explants and cultured them in medium supplemented with 125-µM 2,4-D and 2.5 g/l activated charcoal. Plantlets were successfully produced by the application of NAA (10µM), allowed for normal seedling growth to occur. Control of ethylene and polyamines has been found to improve somatic embryogenesis in coconut. Adkins *et al.* (1998) used cotyledonary slices from coconut embryos and cultured in medium with additives like aminoethoxyvinylglycine (AVG) and silver

thiosulphate (STS), which could reduce ethylene production or polyamines such as spermine, putrescine and spermidine. Somatic embryogenesis was promoted by supplementation with AVG (2μM) or STS (3μM) or by the addition of putrescine (7.5μM) and spermine (1μM). STS also aided somatic embryo proliferation, maturation, and germination.

Use of zygotic embryo culture for germplasm collection, storage and retrieval has been standardized and put in to practice in India (Karun and Sajini, 1994; Karun *et al.*, 1996). Koshy and Kumaran (1997) collected 15 accessions from the Indian Ocean Islands of Mauritius, Madagascar and Seychelles (Anon, 1998) and later, Parthasarathy (2001) used this technique to collect four accessions from Sri Lanka.

Santamaria *et al.* (1999) suggested that sucrose might be important in early stages of coconut embryo cultures, to maintain high chlorophyll concentrations and a high number of chloroplasts. The continuous growth of the resulting plantlets in sucrose containing medium, however, will affect the development of photoautotrophy and in turn affect the performance when transferred to soil. Physiological and biochemical variations in plantlets due to variations in media compositions have been studied (Naresh Kumar *et al.*, 2002a). The establishment of photosynthetic mechanism in *in vitro* development of zygotic embryo-cultured coconut plantlets and during acclimatization had been delineated (Triques *et al.*, 1997a and b; Naresh Kumar *et al.*, 2001). Plantlets also undergo chlorophyll and leaf morphological acclimatization (Ranasinghe *et al.*, 1999). The RUBISCo activity was low in *in vitro* coconut plants just before acclimatization. The ratio of PEPCo to RUBISCo decreased during the *in vitro* development and is an indicator of transition from heterotrophic to autotrophic phase in coconut (Triques *et al.*, 1997 a and b). Later, Naresh Kumar *et al.* (2001) have shown that the embryo cultured coconut plantlets undergo photosynthetic acclimatization with increased PSII efficiency and water use efficiency during plantlet acclimatization process. They also shown that even though the zygotic embryo cultured plantlets initially have less photosynthetic rates, they attain rates similar to those in seedling raised ones in due course of time. Sandoval *et al.* (2003) studied the cell cycle of coconut palm tissues cultured *in vitro* in order to regulate regeneration. Coconut palm is a plant for which it is difficult to monitor the ability of the meristematic cells to actively divide. Cell nuclei were isolated from various types of coconut palm tissues with and without in vitro culture. After the nuclei were stained with propidium iodide, relative fluorescence intensity was estimated by flow cytometry. Characterization of the cell cycle reinforced the hypothesis of a block in the G0/G1 and G1/S phases of the

coconut cells. A time-course study carried out on immature leaves revealed that this block takes place gradually, following the introduction of the material in vitro. Synchronization of in vitro-cultured leaves cells using 60 μM aphidicholin revealed an increase in the number of nuclei in the S phase after 108 h of treatment.

5.2. *Molecular markers:* The use of biochemical and molecular markers in coconut has been a recent one. The biochemical markers like isozymes and molecular markers like Restricted Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Sequence Tagged Microsatellites (STM) are presently being tried. A comprehensive review on the application of molecular markers was first presented by Rohde (1993) and later by Ashburner (1999). Fernando and Gajanayake (1997) have reported protocols for detection of isozyme polymorphism in coconut leaf tissues. They found esterases to be useful for studying the genotypic variations in coconut. Cardena *et al.* (1998) used electrophoretic patterns of leaf peroxidases, endopeptidases and coomassie blue stained proteins in four cultivars and two hybrids. The polymorphism detected fit the expression of two alleles of a dimeric peroxidase, two monomeric endopeptidase and a pair of active and null alleles of a Coomassie blue stained protein. They concluded that the protein markers would broaden the alternatives available to breeders of coconut. Geethalakshmi *et al.* (2000) observed limited polymorphism for esterase and polyphenol oxidase while polymorphism for peroxidase was absent. However, earlier attempts by Meunier *et al.* (1992) failed to achieve any success. The diversity and phylogenetic analysis in 22 populations of Mexican coconut and six imported populations were estimated using 15 enzymatic systems and the allele frequencies in: peroxidase (PER), endopeptidase (ENP), and glucose 6-phosphate dehydrogenase (G6PD). There was very low polymorphism, not more than two alleles per locus. The Wright fixation indexes F(it)=0.62, F(is)=0.40, and F(st)=0.36 indicated low total heterozygosiy within populations suggesting endogamy and genetic drift, and a high diversity among populations due to differentiation between Pacific and Gulf of Mexico coastal populations (Zizumbo Villarreal *et al.*, 2002). The phylogenetic tree with values for genetic distance, indicated three groups on the Pacific coast related to Rennell Tall and Polynesian Tall, and two groups on the coast of the Gulf of Mexico, one related to West African Tall and another related to Mexican Pacific coast populations. This corroborates historical antecedents and morphological and physiological patterns. The Dwarf coconuts related to the Pacific Tall populations, Rennell Tall and

Polynesian Tall. There was no difference between local and imported Dwarf populations (Zizumbo Villarreal *et al.*, 2002). Parthasarathy *et al* .(2004) analysed the diversity using isozyme banding data with 11 isozyme systems in 40 different cultivars and six hybrids and their parents. The cultivars grouped into six clusters. In case of hybrids and their parents, the hybrids clustered intermediate between parents.

Rohde's (1993) preliminary studies have revealed molecular characterization of the nuclear genome, which has provided evidence for the existence of truncated, copia-like repetitive sequences indicating that retro-elements may have played their role in the generation of genetic diversity in coconut. Rohde *et al.* (1995) described a novel approach for the analysis of coconut germplasm by the use of coconut specific primers complementary to the *copia*-like *Eco*RI elements. PCR amplification of spacer regions for a sub-set of tandemly arranged repeats detected polymorphisms, which allowed an analysis of biodiversity within coconut populations. Rohde (1996) has subsequently described Inverse Sequence Tagged Repeat (ISTR) Analysis. Duran *et al.*, (1997) analyzed 48 coconut genotypes using different DNA marker techniques, namely, RAPD, microsatellite primed PCR and ISTR. All three approaches detected a large number of DNA polymorphism among the genotypes and allowed the identification of single genotypes by individual-specific fingerprints. The use of polymorphic microsatellites for assessing genetic diversity in coconut has been gaining popularity (Karp, 1999). CIRAD in collaboration with COGENT developed a set of 14 microsatellite markers with sufficient discriminating power for practical identification of coconut cultivars. These projects culminated in developing standard protocols without the use of radioactive probes as well as development of dedicated statistical software - Gene class 2, adapted to use in the producing countries (Baudouin and Lebrun, 2002). Hautea *et al.* (2000), Perera *et al.* (1999) and Perera (2001) used microsatellites (simple sequence repeat - SSR) to assess the genetic diversity of selected germplasm. The SSR data indicated a high degree of allelic diversity for microsatellite markers within the tall populations. Diagnostic SSR markers were identified for use in hybrid testing and two diagnostic markers were identified for use in hybrid test. Perera *et al.* (2000a) used eight pairs of SSR primers to analyze the genetic diversity in 130 individuals of coconut comprising of 75 tall individuals and 55 dwarf individuals, representing 94 different coconut ecotypes throughout the world. Fifty-one alleles were detected, with an average of 6.4 alleles per locus. Fifty alleles were detected in tall coconuts (talls: mean alleles/locus 6.3) compared with only 26 (mean/locus 3.3) in dwarfs, and the average

diversity value in talls (0.589) was also significantly higher than that in dwarfs (0.348). Using the eight SSRs, they were able to uniquely discriminate 116 of the 130 individuals. A phenetic tree based on D_{AD} (absolute distance) values clustered individuals into five groups, each mainly composed of either talls or dwarfs. Perera *et al.,* (2000 b) also used SSR to study SSR polymorphism. They used 39 coconut specific microsatellite primers developed from an enriched small insert genomic library. Eighteen of those were used to assay Sri Lankan coconuts. The out breeding Tall variety (*typica*) accounted for most of the diversity in contrast to inbreeding varieties, nana (dwarfs) and intermediate (*aurantiaca*) types. Partitioning of the genetic variability revealed that for dwarf and intermediate forms most variation was observed between rather than within forms. In contrast, tall forms exhibited as much variation within as between forms. A reduction in allelic variability was observed in dwarfs compared with talls and the pattern of allelic distributions suggested that Sri Lankan dwarfs were introductions. They used twelve pairs of microsatellite primers to screen a collection of global coconut germplasm. Eighty four alleles were detected in talls as compared to 42 in dwarfs with an average diversity value of 0.703 which was significantly higher than that detected in the dwarf sample (0.374). They concluded that dwarfs are a subset of the tall coconuts and have directly evolved from talls and from 'Niu vai' types of tall (Southeast Asia and Pacific origin). Microsatellite markers have been successfully used to study the genetic diversity in coconut in many laboratories. Meerow *et al.* (2003) analyzed genetic variation within *Cocos nucifera* germplasm collections at two locations in south Florida, representing eight cultivars, using 15 simple sequence repeat (SSR) microsatellite DNA loci. The loci were also used in a parentage analysis of progeny of the 'Fiji Dwarf' variety at both locations. A total of 67 alleles were detected, with eight the highest number at any one locus. These loci identified 83 of the 110 individual palms. Gene diversity of the 15 loci ranged from 0.778 to 0.223, with a mean of 0.574. 'Fiji Dwarf', 'Malayan Dwarf', 'Green Nino' and 'Red Spicata' cultivars resolve as distinct clusters in a neighbor joining tree using modified Rogers distance, while the tall varieties form two aggregates. The highest gene diversity was found in the tall cultivars (H=0.583 cumulatively), and the lowest in the 'Malayan Dwarf' (H=0.202). After the tall coconuts, the 'Fiji Dwarf' was most genetically diverse (H=0.436), and had the largest number of unique alleles. Genetic identity is highest among the 'Malayan Dwarf' phenotypes, and between the tall varieties. The 'Red Malayan Dwarf' is genetically distinct from the 'Green' and 'Yellow Malayan Dwarf' phenotypes, which cannot be distinguished with the SSR loci used. Off-type 'Malayan Dwarf'

phenotypes (putative hybrids with talls) can be identified genotypically. Parentage analyses of 30 'Fiji Dwarf' progeny propagated from five adults surrounded by other cultivars estimate that only 20% of the progeny were out-crossed to the other varieties, while 40-46% were possible self's. This suggests that a seed-production orchard of the variety maintained at reasonable distance from other varieties will likely yield only 'Fiji Dwarf' genotypes. The extent of genetic diversity and the genetic relationships among 94 coconut varieties/populations (51 Talls and 43 Dwarfs) representing the entire geographic range of cultivation/distribution of the coconut was assessed by Perera *et al.* (2003) using 12 pairs of coconut microsatellite primers. A high level of genetic diversity was observed in the collection with the mean gene diversity of 0.647±0.139, with that of the mean gene diversity of Talls 0.703±0.125 and 0.374±0.204 of Dwarfs. A phenetic tree based on DAD genetic distances clustered all the 94 varieties/populations into two main groups, with one group composed of all the Talls from Southeast Asia, the Pacific, west coast of Panama, and all Dwarfs and the other of all Talls from south Asia, Africa, and the Indian Ocean coast of Thailand. The allele distribution of Dwarfs highlighted a unique position of Dwarf palms from the Philippines exhibiting as much variation as that in the Tall group. The grouping of all Dwarfs representing the entire geographic distribution of the crop with Talls from Southeast Asia and the Pacific and the allele distribution between the Tall and Dwarf suggest that the Dwarfs originated from the Tall forms and that too from the Talls of Southeast Asia and the Pacific. Talls from Pacific Islands recorded the highest level of genetic diversity (0.6±0.26) with the highest number of alleles (51) among all the regions.

Konan *et al.* (2007) carried out assessment of genetic diversity of coconut accessions tolerant (Vanuatu Tall and Sri-Lanka Green Dwarf) and susceptible (West African Tall) to lethal yellowing disease. The F_{ST} index revealed that 59.70% of the total allele variability explained differences between the three accessions. Genotypes of West African Tall, susceptible to the lethal yellowing disease, were found to be less clustered genetically to the genotypes of the two tolerant accessions.

The Maypan, a hybrid of Malayan Yellow Dwarf (MYD) and Panama Tall coconut, earlier considered highly resistant, was devastated by an outbreak of lethal yellowing disease in Jamaica. Lebrun *et al.* (2007) used 34 SSR markers to compare the MYD sampled from four locations in Jamaica along with a reference DNA of MYD collected from five different countries *viz.* Ghana, Malaysia, Philippines, Mexico and India to check whether the affected planting material in Jamaica was genetically similar

to the material earlier shown to be resistant to lethal yellowing disease. The results revealed more variation at 34 simple sequence repeat loci in MYD samples from Jamaica than from the rest of the world. About 16% of alleles in Jamaican MYD samples did not match the usual typical MYD genotypes showing that the Jamaican MYD palms were only partially true to type and this heterogeneity may have some undesirable effect on its degree of resistance to lethal yellowing disease.

The extent of genetic diversity in 26 coconut accessions from the Andaman and Nicobar Islands, India was determined using 14 microsatellite markers by Rajesh *et al.* (2008 a). A high level of population differentiation was observed among these (mean F_{ST} of 0.49) confirming the existence of rich genetic diversity of coconut accessions from these Islands. The tall accessions from the Nicobar Islands possessed the majority of rare alleles were observed.

Rajesh *et al.* (2008 b) carried out a study to assess the pattern of diversity in 102 coconut palms representing 10 landraces from three coconut-growing communities of India using 14 simple sequence repeat (SSR) markers. The high mean fixation index (F_{ST}: 0.42) indicated the presence f a high level of population differentiation among the landraces.

A wide range of variability has been observed for morphological characters of coconut populations from Lakshadweep islands. A total of nine accessions collected from Agatti and Kavaratti Islands of Lakshadweep (three Laccadive Micro types, four types of Laccadive Ordinary Tall differing in nut bearing habit, fruit shape and size, one each of sweet husked Kaithathali tall and dwarf type) as part of a germplasm enrichment programme for island coconut populations of India were analyzed using microsatellite markers (Devakumar *et al.*, 2010). The results revealed the existence of high genetic diversity among the coconut populations. In a later study, Rajesh *et al.* (2014 e) analysed the extent of variability and phylogenetic relationships within populations of Laccadive Ordinary Tall (LCT) and Laccadive Micro Tall (LMT) from Amini and Kadmat Islands, Lakshadweep using microsatellite markers. Elliptical type from Amini, which are described as 'Niu Kafa' type, appeared to be distinct from other populations and was phylogenetically related to round or 'Niu Vai' type from Amini. Pear shaped types, the introgressed form, from both the islands showed affinity and appear to have developed as a result of introgression between elliptical and round types. The results of this study revealed the existence of a large extent of variations were also found among individual palms of these distinct types, stressing the importance of selection of LCT

mother palms for hybrid seed production.

Microsatellite markers have also been used for the assessment of genetic purity in coconut hybrids (Rajesh *et al.*, 2012). A set of 50 microsatellite markers were utilized to characterize parental lines Chowghat Green Dwarf (CGD) and West Coast Tall (WCT) used for hybrid coconut production. A panel of informative SSR markers, capable of distinguishing these parental lines, was identified and these markers were successfully utilized in DxT hybrid authentication studies in coconut nurseries. Microsatellite analysis of a large-fruited coconut population from Maharastra State, Vaibhavwadi Tall, indicated that the population was close to the South East Asian coconut accessions and has proximity with dwarf accessions conserved in India (Niral *et al.*, 2013). The probable origin of this type was reported to be another cultivar producing large nuts viz., Borneo Tall.

Rajesh *et al.* (2014 d) carried out analysis of genetic diversity, utilizing microsatellite markers, of Bedakam and Annur ecotypes of coconut from Kerala State, Indian, and compared these ecotypes with predominant West Coast Tall (WCT) populations, from which they are assumed to have originated. The clustering analysis revealed that Annur and Bedakam ecotypes were distinct coconut populations compared to WCT.

Rajesh *et al.* (2014 b) estimated the rate of outcrossing in West Coast Tall (WCT) cultivar of coconut using microsatellite simple sequence repeats (SSR). Two WCT mother palms and their 88 progenies were screened using 15 highly polymorphic microsatellite primers. The percentage similarity between the mother palm and its progenies ranged from 55 to 74 per cent. The results revealed the WCT cultivar to be predominantly out-crossing and indicated that proper sampling and breeding strategies are required to sustain the high genetic diversity found.

Genetic diversity in coconut populations across the entire geographic range was assessed using SSRs and AFLP by Teulat *et al.* (2000). Nagaraju *et al.* (2002) were the first to standardize DNA amplification fingerprinting (DAF) in coconut. They used DAF and amplified fragment length polymorphism (AFLP) markers for studying the phylogenetic relationships among coconut accessions grown in India. The AFLP approach was more efficient as the number of primer combinations detected polymorphic DNA markers were more in contrast to DAF. However the number of polymorphic bands identified using primers selected in both techniques was comparable. The genetic similarities among the accessions were determined. In DAF, out of 300 primers screened, 28 (9.33%) detected

polymorphism producing an average of 5 polymorphic bands while in AFLP 55 (86%) primer combinations generated polymorphic bands (6.42). Dendrogram of the coconut accessions by UPGMA cluster analysis indicated grouping of all the dwarf accessions into one group in both DAF and AFLP analysis.

The use of RFLP and RAPD has been also reported. RFLP markers were used by Lebrun *et al.* (1998a, 1998b and 1999) to study the spread and domestication of coconut using the genetic diversity. They used 289 palms, representative of 26 tall and 16 Dwarf ecotypes originated from major coconut areas. Twenty cDNA probes from oil palm, rice, maize and coconut and one cytoplasmic probe from wheat were hybridized on digested DNA using four restriction enzymes. Based on the molecular polymorphism they defined two main groups of tall coconut palms, originating from South East Asia and Pacific Ocean and another from the Indian subcontinent and West Africa. The cultivars from East Africa and from the Andamans shared markers from both groups, whereas the Panama Tall appeared to be derived from the first one. All the Dwarfs (except Niu Leka) formed a highly homogenous group related to the first group of Talls. Lebrun *et al.* (1999) reported that RFLP analysis is an efficient and powerful technique to obtain a precise picture of coconut diversity and the ways in which it has spread and evolved. Ashburner *et al.* (1997) and later by Wadt *et al.* (1999) described the use of RAPD in coconut. Ashburner *et al.* (1997) studied the diversity in coconut in the South Pacific region. They reported moderate level of genetic diversity although very few RAPD mark-ers were unique to specific populations. Recently Upadhyay *et al.* (2002) screened 100 random primers and found only 53% of the primers amplified coconut DNA and 34% primers detected polymorphism between West Coast Tall and Chowghat Orange Dwarf. Analysis of the genetic distances revealed that all dwarf accessions were grouped together whereas tall accessions showed much heterogeneity. Dewi Hayati *et al.* (2000) used RAPD to analyze genetic diversity of four dwarf populations from East Java. They found that variability of coconut population grown outside East Java was higher than that in East Java since those coconut populations were collected from seeds of open pollinated plants. Randomly amplified polymorphic DNA (RAPD) markers were used to analyze genetic diversity and genetic relationship among coconut accessions using DNA from 81 palms representing 20 accessions, 15 Indian and 5 exotic, with 8 highly polymorphic primers (Upadhyay *et al.*, 2004). The 8 primers yielded 77 markers, with an average of 9.6 markers per primer. The within-accession genetic diversity ranged from 0.057 to 0.196. In general, tall accessions

were more heterozygous as they had higher proportions of polymorphic bands and genetic diversity. The proportion of variation explained by within accession and between accession diversity was 0.58 and 0.42, respectively. Similarly exotic accessions exhibited more variation. Dwarfs from geographically distant regions did not cluster separately. In an earlier study, they analysed fourteen coconut accessions (9 talls, 4 dwarfs and 1 intermediate type) using RAPD markers to establish genetic similarity among some indigenous and exotic coconut accessions maintained in the coconut germplasm centre in Kerala, India (Upadhyay *et al.*, 2002). Of the 100 primers tested, only 54 primers amplified coconut DNA. Thirty-four primers detected at least one polymorphic band between one tall (WCT) and one dwarf (COD) accession. The number of polymorphic bands per primer ranged from 1 to 16. A total of 245 bands were generated by 34 polymorphic primers, of which 116 (47%) were polymorphic. The average number of polymorphic bands per primer was 2.2 (3.4 when only polymorphic primers were considered). Six primers generated 51 bands in 14 accessions, of which 35 (69%) bands were polymorphic. Among tall accessions 50 bands where present, of which 33 (66%) were polymorphic. In contrast, dwarf accessions had 30 bands and only 14 (47%) were polymorphic. The total heterogeneity among 14 accessions was 0.49 whereas that for tall and dwarf accessions was 0.46 and 0.40, respectively. The pairwise genetic distance varied from 0.189 to 0.62. The average genetic distance among dwarf (0.31) was significantly less than that among tall (0.45) accessions. Daher *et al.* (2002) also estimated the genetic divergence among 19 coconut tree populations by random amplified polymorphic DNA. The DNA samples obtained from the leaves of each cultivar were amplified with 24 primers. A total of 127 polymorphic and 61 monomorphic loci were obtained. Six different clusters, possibly heterotic groups, were formed. Group 1 included the dwarf group cultivars. Giant accessions, abbreviated to GBR (Brazilian Giant), formed group 2, except GBRPF, which together with West African Giant (GOA) formed group 4. The most distant accession was the Tonga Giant cultivar (GTG) that did not group with the others and presents potential for hybridization with the six cultivars in the dwarf group cultivars and with the five in the GBR group. Group 3 consisted of GRL, GPY and GRT and Group 5 of GML and GVT. The dendrogram obtained by the nearest neighbour method was in line with the clustering obtained by the Tocher optimization method. The markers used permitted the identification of each of the populations showing that they were genetically different (absence of duplicity). The use of compound samples was effective to investigate the interpopulational genetic diversity.

RAPD markers have also been utilized for identification of tall/ dwarf trait in coconut using a bulked DNA approach (Rajesh *et al.*, 2014e). Screening of tall and dwarf palm bulk DNA with 200 primers revealed a RAPD primer OPBA3 which was able to clearly differentiate both the tall and dwarf bulks. For validation, the primer was used to screen individual tall and dwarf coconut palms representing different geographic regions. The primer was also successfully used to screen the parents and validate hybrids of Dwarf x Tall crosses.

The application of AFLP in coconut has been reported by Perera *et al.* (1998). They generated 322 amplification products from the 42 genotypes with eight pairs of primers (*Eco* RI and *Mse* I). Overall wide variation was detected in the tall (*Typica*) rather than the intermediate (*Aurantiaca*) and dwarf (*Nana*) forms. A hierarchical analysis of molecular variance (AMOVA) was used to quantify and partition levels of variability into between and within form components. They found that for the inbreeding dwarf and intermediate forms most variation was observed between, rather than within forms. In contrast, the outbreeding tall forms exhibited as much variation within as between forms. These observations have important implications for the maintenance and collection of coconut germplasm. Morphologically *Aurantiaca* group is considered to be intermediate between the tall and dwarf accessions. Estimation of genetic relatedness based on AFLP analysis identified the *Aurantiaca* group as being more similar to the dwarf rather than the tall group. In addition, putative duplicate accessions were identified in the *Aurantiaca* group.

The first linkage map on coconut was reported by Rohde *et al.* (1999) using a first population of 52 F_1 plants from the MYD 20 x LAG 07 (Laguna Tall) using ISTR. An initial analysis of this mapping population identified 51 polymorphic ISTR markers, 43 of which could be arranged into 12 linkage groups comprising a total of 542 recombination units. Subsequently Herran *et al.* (2000) and Lebrun *et al.* (2001) constructed linkage map. Herran *et al.*(2000) work was identical to that of Rohde *et al.*'s (1999) using identical mapping population while Lebrun *et al.* (2001) used Rennell Island Tall (RIT) population. They reported the total genome length to be 1971 cM for the RIT map, with 5-23 markers per linkage group. QTL analysis for yield characters in two consecutive sampling periods identified nine loci while three and two QTLs were detected for number of bunches and one and three QTLs for number of nuts. Their study indicated that the co-segregation of markers with these QTLs provides an opportunity for marker-assisted selection. Cardena *et al.* (1999) described the prospects for marker assisted breeding of lethal yellowing resistant

coconuts. The only effective means for controlling LY is replanting with resistant germplasm. Breeding coconuts for any desirable traits is hindered by the long generation time, low multiplication rate, and ineffective clonal propagation of this crop. Additionally, the lack of genotypes adequate for identifying markers linked with LY resistance demands alternative approaches. Cardena *et al.* (2003) identified three coconut populations which could be used for this purpose, and comprised the susceptible West African Tall (WAT), the resistant Malayan Yellow Dwarf (MYD), and a resistant population of Atlantic Tall (AT) plants. This latter material was closely related to WAT, and both of them were distantly related to MYD. The objective of this work was to use those populations for identifying RAPDs associated with LY resistance. RAPDs were considered as associated with that trait if their frequencies were high in MYD and AT, and low in WAT. A total of 82 RAPDs could differentiate the DNA pools from MYD and WAT, and 12 of them appeared at frequencies _0.85 in MYD, and _0.15 in WAT. Five of such markers were in AT at frequencies of 0.80 (-B4570) or 1 (-A11990, -B111140, -AL31160 and -AL7350).

Shalini *et al.* (2007) reported identification of molecular markers with mite resistance in coconut. Mite resistant and susceptible accessions were collected and analyzed using RAPD and SSR primers. Nine SSR and four RAPD primers were identified with mite resistance using single marker analysis. When step wise multiple regression analysis of RAPD and SSR data was done, a combination of five markers could account for 100% of the association with mite resistance.

Mauro-Herrera *et al.* (2007) reported analysis eight cultivars of Florida coconut germplasm using 13 markers derived from WRKY sequences containing single nucleotide polymorphisms (SNP) and one microsatellite. The results obtained through WRKY were comparable to those with microsatellite markers. Even though lower number of alleles were identified with the WRKY-derived markers compared to microsatellite markers, individuals of cultivars 'Red Malayan Dwarf', 'Fiji Dwarf' ('Niu Leka') and 'Red Spicata' were clearly clustered, as reported in an earlier study utilizing microsatellite markers (Meerow *et al.,* 2003). Individuals of 'Green Malayan Dwarf' and 'Yellow Malayan Dwarf' cultivars, however, were resolved with other varieties.

Advances in genomics research have resulted in the development of novel gene-targeted markers. Rajesh *et al.* (2015a) have evaluated a simple and novel marker system, start codon targeted polymorphism (SCoT), for its use as a potential marker system in coconut. Assessment of

genetic diversity in 23 coconut accessions (10 talls and 13 dwarfs), representing different geographical regions, was carried out utilizing 15 SCoT primers. The extent of genetic diversity observed based on SCoT analysis of coconut accessions was comparable to earlier findings using other marker systems. Coconut accessions from the same geographical region clustered together and tall and dwarf coconut accessions clustered distinctly. The results revealed the scope of SCoT markers to be exploited as molecular markers for genetic diversity studies in coconut.

Molecular markers have been successfully used in coconut to study and characterize the pathogens associated with various diseases. Lethal yellowing (LY) disease of coconut palm in Cuba has been reported since the end of the 19th century. To ascertain the presence of phytoplasmas associated with this disease, Llauger *et al.* (2002) took leaf samples from plants showing typical disease symptoms and assayed for the LY agent by the polymerase chain reaction (PCR) using LY-specific primers. Selected PCR amplification products were cloned, sequenced and compared to that of a Mexican LY isolate from the Yucatan region. The results obtained confirm the presence of LY phytoplasma in Cuba. Cuban and Mexican isolates show an overall high degree of sequence similarity with occasional point mutations and small deletions or insertions. Based on these identified genetic differences, LY isolates from the Havana and the Yucatan region cluster together and apart from isolates originating at Maisi in eastern Cuba. Harrison *et al.* (2002) detected DNA of phytoplasmas in lethal yellowing (LY)-diseased palms by a nested polymerase chain reaction (PCR) assay employing rRNA primer pair P1/P7 followed by primer pair LY16Sf/LY16-23Sr. Polymorphisms revealed by HinfI endonuclease digestion of rDNA products differentiated coconut-infecting phytoplasmas in Jamaica from those detected in palms in Honduras, Mexico, and Florida, USA. A three fragment profile was generated for rDNA from phytoplasmas infecting all 21 Jamaican palms whereas a five fragment profile was evident for phytoplasmas infecting the majority of Florida (20 of 21), Honduran (13 of 14) and Mexican (5 of 5) palms. The RFLP profile indicative of Florida LY phytoplasma was resolved by cloning into two patterns, one of three bands and the other of four bands that together constituted the five fragment profile. The two patterns were attributed to presence of two sequence heterogeneous rRNA operons, rrnA and rrnB, in most phytoplasmas composing Florida, Honduran and Mexican LY strain populations. Unique three and four fragment RFLP profiles indicative of LY phytoplasmas infecting Howea forsteriana and coconut palm in Florida and Honduras, respectively, were also observed. By comparison, the Jamaican LY

phytoplasma population uniformly contained one or possibly two identical rRNA operons. No correlation between rRNA interoperon heterogeneity and strain variation in virulence of the LY agent was evident from this study. Cordova *et al.* (2003) detected in the DNA of the lethal yellowing (LY) phytoplasma in 13 of 72 embryos from fruits of four diseased Atlantic tall coconut palms by polymerase chain reaction (PCR) assays employing phytoplasma universal rRNA primer pair P1/P7, nested LY group-specific rRNA primer pair 503f/LY16Sr or LY phytoplasma-specific nonribosomal primer pair LYF1/R1. Phytoplasma distribution in sectioned tissues from six PCR positive embryos was determined by in situ PCR and digoxigenin-11-deoxy-UTP (Dig) labelling of amplification products. Dig-labeled DNA products detected by colorimetric assay were clearly evident on sections from the same three embryos investigated in detail by in situ PCRs employing primer pairs P1/P7 or LYF1/R1. Deposition of blue-green stain on sections as a result of each assay was restricted to areas of the embryos corresponding to the plumule and cells ensheathing it. By comparison, similarly treated embryo sections derived from fruits of a symptomless Atlantic tall coconut palm were consistently devoid of any stain. Presence of phytoplasma DNA in embryo tissues suggests the possible potential for seed transmission which remains to be demonstrated. *Ganoderma* wilt is a serious disease in both coconut and oil palm. Latiffah *et al.* (2002) conducted restriction analysis and sequencing of the ITS regions and 5.8S genes of ribosomal DNA on 53 *Ganoderma* (causing basal stem rot on oil palms) isolates from infected oil palm [*Elaeis guineensis*] and 15 isolates from coconut stumps to determine their relatedness. Restriction patterns of the ITS regions and 5.8S gene using seven restriction enzymes, namely, *HindIII*, *EcoRI*, *BamHI*, *HaeIII*, *MspI*, *TaqI*, and *AluI* did not produce any patterns that could distinguish between *Ganoderma* isolates from infected oil palm and coconut stumps. Variations of restriction patterns were observed within and between the two groups of Ganoderma which showed that the isolates were genetically heterogeneous. Based on the dendrogram from cluster analysis of the restriction patterns, the *Ganoderma* isolates from infected oil palm and coconut stumps were clustered together, indicating a close relationship. Phylogenetic analysis of the nucleotide sequence of the ITS regions and 5.8S gene of 12 *Ganoderma* isolates using parsimony and distance methods also showed that the two groups of *Ganoderma* did not cluster separately. Based on the present study, the *Ganoderma* isolates from infected oil palm and coconut stumps were indistinguishable and closely related which suggests that the coconut stumps in oil palm plantings may have an important role in disease development.

5.3. Gene cloninig

In spite of the agronomic importance of coconut, studies of germplasm assessment have depended on morphological and agronomical traits and recently, molecular markers. Molecular biology techniques have been used scarcely in assessment of genetic resources and for improvement of important agronomic and quality traits in coconut, which mainly is due to the dearth of available sequence information. With the advent of next generation sequencing technologies, ttranscriptome sequencing has turned out to be the technique of choice for large scale gene discovery. Massively-parallel sequencing of RNA (RNA-Seq) has resulted in a dramatic increase in the RNA sequencing output, in addition to enabling global measurement of transcript abundance, in comparison to earlier cloning techniques.

To explore the molecular mechanisms involved in compatible and incompatible interactions with respect to coconut root (wilt) disease, Rajesh *et al.* (2013) carried out transcriptome profiling of susceptible and healthy Chowghat Green Dwarf (CGD) palms. Many of these differentially expressed transcripts were primarily involved in defense responses, signalling pathways, cellular transport and other metabolic processes. The resources generated in this study provided new insights into the interaction of coconut palms with root (wilt) disease pathogen.

Fan *et al.* (2013) applied RNA-seq technology and de novo assembly to gain a global overview of coconut transcriptome from mixed tissue samples (spear leaves, young leaves and fruit flesh). Using Illumina sequencing, they obtained 54.9 million short reads and conducted *de novo* assembly to obtain 57,304 unigenes (average length of 752 base pairs), out of which 23,168 were mapped into 215 KEGG pathways, including galactose metabolism, plant-pathogen interaction and plant hormone signal transduction pathways. In addition, the study revealed 347 unigenes which were involved in fatty acid synthesis and metabolism.

Huang *et al.* (2014) reported *de novo* transcript assembly from RNA-seq data and analysis of gene expression in leaves and seed tissues (embryo and endosperm) of green dwarf variety. Assembly of sequencing data for each tissue resulted in a total of 58,211 unigenes in embryo, 61,152 in endosperm, and 33,446 in leaf. KEGG pathway analysis identified 138, 138, and 139 pathways, respectively, in transcriptomes of embryo, endosperm, and leaf tissues. Homology searches were undertaken to identify putative homologs of factors required for RNA-directed DNA methylation in coconut. The results obtained suggested that RNA-directed DNA methylation was important during coconut seed development,

especially in maturing endosperm.

Nejat *et al.* (2015), using RNA-Seq technique, evaluated the whole transcriptome profiles of naturally infected leaves of Malayan Red Dwarf in response to yellow decline phytoplasma. The results obtained from this study revealed that more genes were down-regulated in response to phytoplasma infection than those being upregulated. Of the 39,873 differentially expressed unigenes, 21,860 unigenes were suppressed and 18,013 were induced following infection. Genes associated with defence signalling against biotic stimuli were significantly overexpressed in leaves of infected palms. Genes differentially expressed included those invovled in cell rescue and defence, cellular transport, oxidative stress, hormone stimulus and metabolism, photosynthesis reduction, transcription and biosynthesis of secondary metabolites. A core set of genes associated with defence of coconut in response to phytoplasma attack was proposed.

RNA-Seq has also been utilized to generate the transcriptome of leaf samples of coconut root (wilt) disease-resistant cultivar Chowghat Green Dwarf (Rajesh *et al.*, 2015). Comprehensive bioinformatics analysis identified 243 resistance gene analog (RGA) sequences, comprising six classes of RGAs. Phylogenetic analysis of deduced amino acid sequences revealed that coconut NBS-LRR type RGAs were classified into distinct groups based on the presence of TIR or CC motifs in the N-terminal regions. The results of this study generated a sequence resource for development of RGA-tagged markers in coconut, which would aid mapping of disease-resistant candidate genes.

Transcriptome analysis (RNA-Seq) of coconut embryogenic calli, derived from plumular explants of West Coast Tall cultivar, was undertaken on an Illumina HiSeq 2000 platform (Rajesh *et al.*, 2015b). They obtained 40,367 transcripts which showed significant BLASTx matches with similarity greater than 40 % and E value of d"10^{-5}. Fourteen genes known to be involved in somatic embryogenesis were identified and validated using RT-qPCR.

The above review would amply show the effort that has been put into coconut biotechnology. Unfortunately, in India, coconut biotechnology has been carried out only at ICAR-CPCRI at present. If one goes by the history, there were nearly a dozen laboratories involved in coconut biotechnology in eighties. When it was discovered that coconut biotechnology is a difficult one, many laboratories stopped working on it. Harries (1999) rightly stated thus " A devil's advocate, asked to say if clonal coconuts really do have any use, would have to admit that the rate

of progress has been disappointingly slow. The early aims, to clone high yielding individual palms as farm plating material, are now seen to be naïve. Academic studies may have generated higher degrees for research scientists but they have not spawned the financially successful industries enjoyed by some crops". But, recent developments may prove Harries wrong - may be that is my holy intention.

5.4. ***In vitro conservation:*** Coconut is a recalcitrant species and the nuts do not undergo maturation drying and are shed at relatively high moisture content (Parthasarathy, 1999). One of the earliest reports of cryopreservation coconut embryos was reported by Chin *et al.* (1989). They found that embryos cryoprotected with 10% DMSO showed the highest percentage survival after cryopreservation followed by 10% glycerol. Earlier Bajaj (1984) reported only elongation of whole embryos or proliferation of the cut ends of transverse halves of the embryos after cryopreservation and he did not observe normal development. He used 7% DMSO and 4% sucrose as cryoprotectants and the percentage of survival was low (17 - 25%). Karunaratne *et al.* (1985) reported one of the earliest attempts to preserve the coconut embryos in culture in a dormant state. They devised a special survival medium, which suppressed the growth of embryos for a period of 5 months. Assy-Bah and Engelmann (1993) found that immature embryos of coconut (7 to 8 months after pollination) can withstand rapid freezing in liquid nitrogen after 4 hours of pregrowth on a semi solid medium containing 600g/l glucose and 10 to 15% glycerol or sorbitol. In these conditions, survival ranged from 10 to 43% and one embryo developed into a rooted plantlet, 2.5 months after freezing. While in a later study Assy-Bah and Engelmann, 1993, observed mature embryos (10 - 12 months after pollination) of four varieties of coconut could withstand cryopreservation in liquid nitrogen and develop into plants. Pretreatment consisted of a 4-hour desiccation in the air current of a laminar flow cabinet followed by a 11 to 20 hour culture on a medium containing 600g/l glucose and 15% glycerol. They carried out freezing and thawing with recovery rates between 33 and 93% of frozen embryos, depending on the variety. The same workers (Assy-Bah and Engelmann, 1993) subsequently developed optimal conditions for the medium term conservation of zygotic embryos. After 6 months of storage on a medium devoid of sucrose and containing 2g activated charcoal/l, 100% of the embryos developed into whole plantlets within five months of transfer to the recovery medium. After a 12-month storage period on medium containing 15g/l sucrose and devoid of activated charcoal, 51% of the embryos germinated within two months after transfer to the recovery medium. The presence of sucrose in

the storage media has been reported to initiate the embryonic response to cellular expansion and elongation as well as cell division of the epidermal layer to keep pace with the expanding tissues (Mkumbo and Hornung, 1997). Engelmann *et al.* (1995) studied the factors affecting the cryopreservation of coconut embryos. They found that embryos should be used only when they are in an optimal physiological state as regards their maturity and metabolic status. Modifications of recovery conditions can greatly increase the survival rate of zygotic embryos.

Sajini *et al.* (2011) carried out experiments to study the effect of pre-culture conditions, vitrification and unloading solutions on survival and regeneration of coconut zygotic embryos after cryopreservation. Out of the seven plant vitrification solutions studied, PVS3 was found to be the most effective for regeneration of cryopreserved embryos. The optimal protocol standardized comprised of pre-culture of embryos for three days on medium with 0.6 M sucrose, PVS3 treatment for 16 h, rapid cooling and rewarming and unloading in 1.2 M sucrose liquid medium for 1.5 h. About 70-80% survival (corresponding to size enlargement and weight gain) was observed with cryopreserved embryos and 20-25% of the plants regenerated (showing normal shoot and root growth) from cryopreserved embryos were established in pots.

A protocol for cryopreservation of coconut pollen was reported by Karun *et al.* (2014). Pollen of two coconut varieties (West Coast Tall and Chowghat Orange Dwarf) was desiccated to 7.5 % moisture content (FW) and cryopreserved by direct immersion in liquid nitrogen. Germination and vigour of cryopreserved pollen were was found to be higher compared to that of oven dried and non-cryopreserved pollen. Normal seed set was observed in COD and WCT palms using pollen cryostored for 6 months and 4 years respectively. These results demonstrated the possibility of long term conservation of coconut germplasm by establishing pollen cryobanks.

Recently, a large number of basic studies on the physiological and biochemical aspects of somatic embryogenesis and regeneration have been published. Based on the response of coconut callus to somatic embryogenesis induction medium (SEI), Magnaval *et al.* (1997) observed three types of response, namely, the traits that were modified by SEI condition and varying over time; the second type of response corresponded to traits modified by the SEI condition but constant over time and the third type of response corresponded to traits unchanged by the SI condition and over time. They studied the specific nutritional requirement of coconut

calli during these phases. In another study by the same group (Magnaval *et al.*, 1995), they classified the calli into five groups based on their amino acid composition by a clustering method. Dussert *et al.* (1995) have presented a detailed study on nutrient uptake and growth of *in vitro* coconut callus. Another aspect of research worth mentioning is the photosynthetic ability of *in vitro* grown coconut plantlets. Triques *et al.* (1997a) studied various photosynthetic parameters using complementary approaches. Transmission electron microscopic studies have revealed a complete ultrastructural organization of chloroplasts in plantlets at the end of the *in vitro* culture process (six weeks under light). Studies by Rival *et al.* (1999) have proved that coconut belongs to the class of plants in which the *in vitro* grown leaves can contribute to autotrophy and then play an active part in acclimatization as indicated by the dramatic decrease in the PEPC/ RUBISCo activity ration and the increase in the photochemical activity of PSII.

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