Breeding of Horticultural Crops Vol. 1 -Part B: Plantation Crops (2017) : 569-589
Editors : V. A Parthasarathy, C. Aswath, K. Nirmal Babu & R. Senthil Kumar
Today & Tomorrow's Printes and Publishers, New Delhi - 110 002, India

ARECANUT

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Arecanut (*Areca catechu* L.) or betelnut is an extensively cultivated tropical palm and the dry kernel is used mainly for masticatory purpose in India and West and South East Asia. The major arecanut growing countries are: India, Sri Lanka, Bangladesh, Nepal, China, Myanmar, Bhutan, Thailand, Malaysia, Indonesia and Vietnam. The present production of arecanut in the world is 1.22 million tonnes from an area of 0.90 million hectares. In India, arecanut is cultivated in about 4.51 lakh ha with a production of 7.47 lakh tonnes of nuts with the productivity of 1659 kg ha^{-1}. Arecanut is predominantly cultivated in traditional states like Karnataka, Assam, Kerala, Maharashtra, West Bengal and Andaman and Nicobar group of Islands and it's cultivation is spread to non-traditional areas in Karnataka and Tamil Nadu. Arecanut is providing livelihood security for millions of people in rural and urban areas.

1. History, origin and distribution

The origin of arecanut is not exactly known. There are no fossil remains of the genus *Areca*, but the fossil records of closely related genera indicate its presence during tertiary period. The maximum species diversity (24 species) and other indicators suggest its original habitat in Philippines, Malaysia, and Indonesia (Raghavan, 1957; Bavappa, 1963). According to Watt (1889) it is a native of Cochin China and Malay Peninsula. Beccari (1919) described four cultivars and nine species of areca from Philippines.

Although it is not precisely known as to when arecanut found its way to Indian subcontinent, several evidences about its antiquity exist (Mohan Rao, 1982). Arecanut is mentioned in various ancient Sanskrit scriptures (650-1300 BC); and its medicinal properties were known to a famous Scholar Vagbatta (4th century A.D.). One of the well-known Ajanta caves in central India (200 B.C. to 900 A.D.) has one exquisitely painted arecanut palm providing a backdrop to Padmapani Budha. According to Furtado (1960) one of the earliest references to arecanut was in 1510 A.D.

The abundant use of arecanut in chewing and religious functions was indicated even during the times of Aryans in India.

2. Botany

Martius (1832-1850) was the first to attempt defining the limits of the genus, *Areca*. But the attempts were not satisfactory, as they were not based on real affinities. Later various species, grouped under *Areca,* were separated into different genera, based on nature of albumen, position of ovule, distribution of flowers, and limited the genus to close relatives of the type of the genus, *Areca catechu* (Blume, 1836). Furtado (1933) described the limits of the genus *Areca* and its sections. A list of *Areca* species and their geographical distribution is given in Table 1. Blatter (1926) described the generic characters of genus *Areca.* Detailed morphology, floral biology and embryology of arecanut have been described (Murthy and Pillai, 1982). The arecanut palm is a graceful, erect and unbranched palm reaching a height of upto 18-20m. The stem has scars of fallen leaves in regular annulated forms. The girth of the stem depends on genetic constitution, soil condition and plant vigour. The arecanut palm has an adventitious root system. The crown of an adult palm contains 7-12 leaves. The leaves are pinnatisect and consist of a sheath, a rachis and leaflets. The leaf sheath completely encircles the stem. It is about 54 cm in length and 15 cm in breadth. The average length of leaf is 1.65 m, which bears about 70 leaflets. The leaflets are 30.0 to 70.0 cm in length and 5.8 to 7.0 cm in breadth depending on the position of the leaf.

Arecanut is monoecious with both male and female flowers occurring on the same spadix. It is cross-pollinated (Bavappa and Ramachander, 1967). The male phase lasts for 25-46 days. Female flowers are cream coloured and turning green within a week. The female phase extends up to 10 days. The stigma remains receptive up to 6 days (Murthy and Bavappa, 1960). Pollen is generally carried by wind.

Table 1. Distribution of Species of Areca

Country	Species
India	*A. catechu, A. triandra*
Andamans, India	*A. catechu, A. laxa*
Sumatra	*A. catechu, A. triandra, A. latiloba*
Sri Lanka	*A. catechu, A. concinna*
Malaysia	*A. catechu, A. triandra, A. latiloba, A. montana, A. ridleyana*
Borneo	*A. catechu, A. borneensis, A. kinabaluensis, A. arundinacea, A. bongayensis, A. amojahi, A. mullettii, A. minuta, A. furcata*

Java	*A. catechu, A. latiloba*
Celebes	*A. celebica, A. oxicarpa, A. paniculata, A. henrici*
Australia	*A. catechu, A. alicae*
Solomon Islands	*A.niga-solu, A. rechingeriana, A. torulo, A. guppyana*
New Guinea	*A. congesta, A .jobiensis, A. ladermaniana, A. macrocalyx, A. nannospadix, A. warburgiana*
Philippines	*A. catechu, A. hutchinsoniana, A. vidaliana, A. costulata, A. macrocarpa, A. parens, A. caliso, A. whitfordii, A. camariensis, A. ipot*

3. Species and cultivars

Several cultivars have been recognized in Karnataka (Rau, 1915) and Philippines (Beccari, 1919) based on fruit and kernel. Cultivars can be separated on the basis of stomatal characters, size of nuts, leaves shed, female flowers, nut size (Bavappa, 1966; Bavappa and Pillai, 1976). A collection of five species *viz.*, *A. catechu*, *A. triandra*, *A. macrocalyx*, *A. normanbyii* and *A. concinna* and two genera *Actinorhytis calapparia* and *Pinanga dicksoni* are available at the Regional Station, Vittal (Anuradha, 1999). The germplasm holding now consists of 113 accessions (Ananda, 1999). Among these, 23 exotic accessions representing different species were introduced from countries *viz.*, Fiji, Mauritius, China, Sri Lanka, Indonesia, Saigon, Singapore, British Solomon Islands and Australia. Besides, about 90 collections represent germplasm from explorations made in different arecanut growing states of India. About 39 accessions have been described based on descriptors.

There is wide range of variations in fruit characters, stem height, internode length and leaf size and shape. The nuts in Malnad parts of Shimoga and Chikmagalur districts are small in size whereas in North Kanara and Ratnagiri they are bigger (Murthy and Bavappa, 1962). There are also wide variations in yield, earliness in bearing, fruit number/bunch, quality, and dwarfness.

The exotic and indigenous collections are under evaluation since 1957 for morphology, nut characters and yield attributes (Ananda, 1999; Bavappa and Nair, 1982). Yield evaluation has resulted in release of four high yielding cultivars of which three are selections from exotic collection. The characteristics of these varieties have been described (Ananda and Thampan, 1999; Nampoothiri *et al.*, 1999; Table 3). Among the exotic collection, cultivar 'VTL – 3' introduced from China was released and named as 'Mangala' (Bavappa, 1977). This cultivar had earliness in bearing, more number of female flowers, high yield and lesser stem height as

compared to other accessions. Other varieties released for cultivation are 'Sumangala' and 'Sreemangala' that are introductions from Indonesia and Singapore respectively. High yield potential was observed in one of the indigenous collection from West Bengal and it was released as 'Mohitnagar' variety. A comparative yield trial involving five high yielding varieties of arecanut *viz.*, Mangala, Sumangala, Sreemangala, Mohitnagar and Thirthahalli local was studied at Thirthahalli to identify suitable variety for cultivation in the Malnad (hills) region of Karnataka (Ananda *et al.*, 2000). 'Mangala' registered significantly lower height with high percentage of flowering palms. 'Mangala' performed better than other varieties with highest yields in the initial years of bearing closely followed by 'Thirthahalli local' while 'Sreemangala' recorded comparatively lower yields. Five arecanut cvs (VTL-3, VTL-11, VTL-12, VTL-13 and VTL-17) were assessed for increasing production/unit area. The mean tree height, number of leaves produced/tree, girth at collar 45 months after planting, time taken for first flowering, nut weight and processing out-turn were recorded. Cultivars VTL-3, VTL-12 and VTL-13 were considered the most productive (Thangaraj *et al.*, 1980). Data on nut weight and nuts/tree were obtained from two experiments, one involving 13 varieties of *Areca catechu* over eight years and the other 11 varieties over nine years. The three highest-yielding varieties proved suitable when planted in favourable environments only. Two others, with fairly high yields, were considered stable in all environments (Natarajan *et al.*, 1982).

Bavappa (1974) recorded morphological, anatomical and yield characters for 13 cultivars of *A. catechu* and four ecotypes of *A. triandra* during the years 1963, 1966 and 1972. The analysis of variance of the results obtained in 1963 showed that the differences between cultivars are highly significant for all the six morphological characters. A combined analysis of the data for two years for 24 common characters recorded during 1967 and 1972 also revealed significant interaction between cultivars for all characters. A significant interaction between years and cultivars was seen for height, girth, internodal distance, number of bunches and inflorescences on the palm, length and breadth of leaf sheath, length and volume of nut and length, breadth, weight and volume of kernel.

4. Genetics and Breeding

The chromosome number of *Areca catechu* L. was first determined and reported by Venkatasubban (1945) as 2n=32. The chromosome number of the species was later confirmed by Sharma and Sarkar (1956), Raghavan and Baruah (1958), Abraham *et al.*, (1961) and Bavappa and Raman (1965).

A chromosome number of 2n=32 reported by Darlington and Janaki Ammal (1945) for *A. triandra* Roxb was later confirmed by Sharma and Sarkar (1956) and Bavappa and Raman (1965). Nair and Ratnambal (1978) determined the meiotic chromosome number of *A. macrocalyx* Becc as n=16. Sharma and Sarkar (1956) described meiotic abnormalities such as non-disjunction, lagging chromosomes, univalents and pentads were reported in *A. catechu.* Bavappa and Raman (1965) studied meiosis of four ecotypes of *A. catechu.* Abnormalities like univalents at diakinesis and metaphase I, non-synchronisation of orientation, clumping, delayed disjunction, chromosome bridges and laggards at anaphase I and II, chromosome mosaics and supernumerary spores were observed.

Sharma and Sarkar (1956) found that the meiotic division was quite normal in *A. triandra* except for the presence of 14 and 18 chromosomes occasionally at metaphase II. Bavappa and Raman (1965) also reported regular meiotic division in *A. triandra.* There was intra-cultivar variation in meiotic behaviour of *Areca* (Bavappa, 1974; Bavappa and Nair, 1978). While normal bivalent formation was observed in some palms, others had maximum association of hexavalents, octovalent and even decavalent. Abnormalities like bridges and laggards, disorientation of chromosomes at anaphase I and anaphase II were also reported in this species. Intra-palm variation in chromosome numbers exist in the pollen mother cells of *A. catechu, A. triandra* and their hybrids. Cytomixis to an extent of 39% seemed to have contributed to this abnormally. In spite of high degree of multivalents in *A. catechu*, pollen fertility was very high. The possibility of the frequency of multivalent formation and disjunction being under genotypic control and being subject to selection was suggested (Bavappa and Nair, 1978). Partial desynapsis of chromosomes occurs at diakinesis in *A. triandra* and *A. catechu* × *A. triandra* hybrids (Bavappa, 1974). Desynapsis observed at diakinesis was followed by an increase in pairing at metaphase I as reflected by the frequency of bivalents in *A. triandra* and *A. catechu* × *A. triandra* hybrids. This was attributed to distributive pairing, a mechanism that has possibly been adopted for ensuring regular segregation of chromosomes. The extent of desynapsis was higher in the F1 hybrids of *A. catechu* and *A. triandra* as compared to *A. triandra*, suggesting that the genetic mechanism controlling this character may be dominant. The large number of univalents observed in the hybrid, as compared to *A. triandra* parent, has been attributed to reduce homology of the parental chromosomes (Bavappa and Nair, 1978). Two pairs of short satellited chromosomes in the somatic chromosome complement of *A. catechu* were observed (Venkatasubban, 1945). Three pairs of long

chromosomes, six pairs of medium sized chromosomes and seven pairs of short chromosomes were observed (Sharma and Sarkar, 1956) in *A. catechu*. They categorized the chromosomes into seven groups based on their morphology and relative length. Two pairs of long chromosomes next to the longest were found to have secondary constrictions. They also observed that the chromosomes of *A. triandra* were longer than those of *A. catechu*. Bavappa and Raman (1965) found the chromosomes of *A. catechu* and *A. triandra* to differ in size, total chromatin length, position of primary and secondary constrictions and number and position of satellites. Based on the assumption of Sharma and Sarkar (1956) that gradual reduction in chromatin matter had taken place in the evolution from primitive to advanced forms of different genera and tribe of Palmae. Bavappa and Raman (1965) considered *A. catechu* as more advanced than *A. triandra*.

The chromosome morphology of a few cultivars of *A. catechu* from Assam was reported (Raghavan, 1957). Minor variation in structure and length of individual chromosomes, total length of the complement and position of constrictions among the types was noted. On the basis of morphology, he recognised nine groups in the somatic chromosomes of the cultivars.

Studies on the karyotypes of eight cultivars of *A. catechu* and four ecotypes of *A. triandra* (Bavappa, 1974; Bavappa *et al.*, 1975) revealed considerable differences in their gross morphological characteristics. The karyotypes of the *A. triandra* ecotypes showed a higher frequency of sub median and median chromosomes as compared to *A. catechu*. A classification of the karyotype of the two species according to the degree of their asymmetry which recognizes three grades of size differences and four grades of asymmetry in centromere position (Stebbins, 1958), showed that karyotypes 1B, 2A, 2B and 3B are represented in *A. catechu* cultivars and only 1A, 2A and 2B are represented in the ecotypes of *A. triandra*. Even within the same cultivar of *A. catechu*, two different types of asymmetry in karyotypes were observed. There was no such variation in *A. triandra* ecotypes. Evidently *A. triandra* has a more symmetrical karyotype than *A. catechu*. It was concluded that delineating the cultivars of *A. catechu* on the basis of standard karyotype is rather difficult. The fact that *A. catechu* has lesser chromatin matter and an asymmetrical karyotype compared to *A. triandra* shows that the latter is more primitive.

The hybridization programme was initiated with the objectives of exploiting the existing variability in the *Areca* germplasm (Bavappa and Nair, 1982). The main concerns were to evolve high yielding, regular bearing, high quality and semi-tall ideotypes. Interspecific hybrids of *A.*

catechu x *A. triandra* had only one stem as in *A. catechu* indicating dominance of this character (Bavappa, 1974). The hybrids mostly equaled the parents in internodal length. They also exhibited hybrid vigour for a number of characters like number of male flowers, female flowers, spadix length and stem girth.

The tall nature of the palm hinders various operations like spraying and harvesting and is quite labour intensive. One of the major thrusts in the research on breeding in arecanut has been to induce dwarfness. A natural dwarf mutant was identified and was named 'Hirehalli Dwarf ' (Naidu, 1963). This however has low yields coupled with very poor quality nuts not suitable for chewing. An attempt was made to cross the high yielding varieties with Hirehalli Dwarf to exploit the dwarfing nature (Ananda, 1999; Nampoothiri *et al.*, 1999). Maximum dwarfs and intermediates were recovered from crosses Sumangala x HirehalliDwarf, Mohitnagar x Hirehalli Dwarf and Mangala x Hirehalli Dwarf among the twelve hybrids crosses made. Hirehalli Dwarf x Sumangala hybrid was also found to be promising with a yield of 2.65 kg/ palm dry nuts during first year of yielding.

5. BIOTECHNOLOGY

Biotechnological research in arecanut is still in infancy. During the last ten years, research has been initiated in certain laboratories which are reported below.

5.1. Tissue culture

Research on tissue culture of arecanut has been reported only during the last ten years. Karun *et al.*, (2004) reported a protocol using leaf and immature inflorescence of adult palms as a explant. They found Picloram to be the suitable callogenic agent. Initial standardization was done on leaf explants excised from one year old seedling and later modified for immature inflorescence sampled from adult palm. The protocol was also tested with different arecanut varieties *viz.*, Mangala, Sumangala and Mohit Nagar; the basal medium used was MS. Picloram was found to be the most suitable callogenic agent for both types of explants as well as for varieties tried Serial transfer of explants from high to low auxin concentration was essential for sustained growth of callus and somatic embryo induction. Somatic embryogenesis was achieved in hormone-free MS medium. Somatic embryo was germinated in MS medium supplemented with cytokininsand 20 μM BA was found to be best. No variation was noticed for callus initiation and somatic embryogenesis and plantlet development

in different varieties except for period of culturing. For rapid germination of somatic embryo, MS liquid medium supplemented with 5 µM BA was used. Plantlet with 2-4 leaves and fairly good root system were weaned using sand: soil (5:1) potting mixture. These plantlets were field planted during 2006 at CPCRI (RS) Vittal for field evaluation. This protocol has been applied for mass multiplication of field resistant Yellow Leaf Disease (YLD) palms (Karun *et al.*, 2005). Direct and indirect somatic embryogenesis from inflorescence explants of areca palms was reported by Radha *et al.*, (2006). However the numbers of direct somatic embryos formed were very less compared to indirect somatic embryos. The effect of various cytokinins *viz.*, TDZ, BA, Kinetin , 2i-P and Zeatin on growth and maturation of direct somatic embryos were studied and it was found that TDZ was essential for maturation and conversion of somatic embryos into complete healthy plantlets (Radha *et al.*, 2008). Mass multiplication was also achieved through indirect secondary somatic embryogenesis.

Mathew and Philip (2000) first reported the protocol for *in vitro* propagation *via* direct adventitious shoot bud differentiation from embryo explants. Results obtained with excised embryos of *A. catechu* (cv. 'Kasargodan' and 'Mangala') grown on Murashige & Skoog's medium, White's medium and Branton & Blake's (BB) medium with permutations and combinations of auxins and cytokinins showed that activated charcoal, 2,4-D and high levels of phosphate in BB medium were critical for the differentiation of additional shoots from the cotyledon. Wang *et al.*, (2003a) developed a protocol for plantlet formation through shoot formation from callus of arecanut. Greenish soft callus was formed from shoot tip explants within four weeks, when cultured on Gelrite-gelled MS basal medium supplemented with BA in combination with TDZ. The highest percentage of callus formation (100%) was found on the medium supplemented with 0.2 mg l^{-1} BA and 0.2 mg l^{-1} TDZ. During subculture on the same medium for callus induction, most of calli proliferated and 50-60% formed shoots. About 90% of shoots formed roots on BM containing 0.1 mg l^{-1} NAA after four weeks in culture. Regeneration of plantlets from shoot tips *via* primary callus production and a two-step process of organogenesis, required about 20 weeks.

Wang *et al.*, (2003 b) obtained plant regeneration through somatic embryogenesis from zygotic embryo-derived callus of arecanut. An *in vitro* culture procedure was established for somatic embryogenesis and plant regeneration from callus cultures. Segments of zygotic embryos were cultured on Murashige and Skoog basal medium supplemented with dicamba (9.05, 18.1, 27.15, and 36.2 µM). After 7-8 week in darkness,

wounded regions of explants formed callus with yellow, soft, glutinous structures. Proliferation and maintenance of callus was on the same dicamba-containing medium. With regular subculture every 8 weeks, the callus showed pale yellow, compact and nodular structures. During subculture, somatic embryos were formed spontaneously from nodular callus tissues within 2-4 months. The embryos developed into plantlets after 10 weeks of culture on basal medium free of plant growth regulators. After subculturing every month for 3 months, the plantlets were transferred to containers for acclimatization in the greenhouse. The survival rate was 24%.

5.1.1. Applications

One of the major production constraints of arecanut is the devastating Yellow Leaf Disease (YLD). This serious malady is prevalent in Kerala and Karnataka states. All the cultivars have been reported to be susceptible for this disease but few South Kanara Local cultivars are found to be field resistant. Surveys conducted in the hot spot regions revealed that healthy palms with good yields are available in the midst of the severely disease affected trees. These healthy palms are being used for resistant breeding programme. The tissue culture protocol developed at CPCRI was used for mass multiplication of field resistant Yellow Leaf Disease (YLD) palms. The field evaluation of these plants have been taken up in three villages *viz.* Gunadka, Balambi, and Nadubettu at Suliya Taluk of Dakshina Kannada District of Karnataka State. The performance of the tissue cultured plantlets in the YLD hot spot region was found to be satisfactory and comparable with seeding raised palms (Karun *et al.*, 2005).

Currently the arecanut tissue culture protocol developed at CPCRI has also been applied for mass multiplication of dwarf arecanut (variety 'Hirehalli') and its hybrids. 'Hirehalli Dwarf", a natural mutant identified in 1963 for its short stature, is a good genetic source for arecanut improvement. The main features of the dwarf are the complete suppression of the internodal space and erect crown shape. This dwarf has been used in all the major hybridization programmes of CPCRI. The identification of hybrids with dwarfness and high yielding potential will benefit areca by way of increased returns and reduced cost of various cultural operations. The breeding programme at the Central Plantation Crops Research Institute Regional Station at Vittal, Karnataka have carried out crossing programme involving various combinations of a local dwarf with five high yielding varieties and have succeeded in developing three promising hybrids. *In vitro* plantlet development could be achieved from inflorescence explants of these hybrids.

5.1.2. Embryo rescue

In vitro germination of excised mature embryos of arecanut was reported by Ganapathi *et al.*, 1997. Mathew and Philip (2000) reported the protocol for *in vitro* propagation *via* direct adventitious shoot bud differentiation from cotyledon explants. Karun *et al.*, (2002) reported an *in vitro* germination technique for rapid germination of arecanut embryos. Seven month old embryos collected from four varieties 'Mangala', 'Sree Mangala''Sumangala' and 'South Kanara Local' were cultured *in vitro* on three agar gelled hormone free medium *viz.*, Eeuwen's Y3 medium, ½ strength MS and full MS. Embryos cultured in Y3 medium supplemented with sucrose 3 % showed maximum germination (93.3 %). *In vitro* plantlets after eight weeks in germination medium were transferred to liquid medium containing reduced level of sucrose (1.5 %) for proper development of roots and expansion of leaves. Fully developed plantlets with minimum varietal difference with respect to germination was also recorded and showed that the varieties 'Sumangala' and 'South Kanara local' were more responsive.

At present, arecanut germplasm is conserved mainly in field gene banks which are exposed to climatic and biological hazards and are costly to maintain over a large area. Being a highly cross pollinated and recalcitrant species, cryopreservation of somatic embryo is the only option for long term conservation of arecanut genetic resources.

5.1. 3 Cryopreservation of arecanut pollen

Areca is a genus of about 76 species of palms in which *Areca catechu* L. is the only cultivable species. It is an allotetraploid palm with chromosome number, 2n =32 and highly cross pollinated. Being a high valued commercial crop, its contribution in terms of livelihood, employment and income to the national economy is significant. As a perennial tree crop arecanut germplasm is maintained in the field gene bank. The arecanut palm is monoecious and its pollen remains viable for 8-9 hr under normal conditions. Increased longevity of pollen, from 15 to 21 days by storing in desiccators at room temperature was reported by Bhat *et al.*, (1962). Since pollen is a useful source of diverse alleles within genepool, storage of pollen is highly essential germplasm conservation as well as for hybrid seed production and also for assisted pollination. Pollen cryopreservation is the only larger scale, long term option for the *ex situ* conservation of arecanut pollen.

Studies were conducted for standardization of procedure for pollen

collection, its desiccation, germination media, and effect of temperature for *in vitro* germination, pollen cryopreservation and its viability and fertility. The fully matured male flowers, which had just opened as well as that are expected to open (white colored perianth) the next day, were collected and dried in at ambient temperature for 24 hours. The pollen was collected by sieving the dried male flowers. For selecting the media for pollen germination, sucrose at various concentrations (2.5 %, 3 %, 4 %) along with agar (1%), gelatin (1%) and boric acid (0.01%) were studied. Fresh as well as desiccated pollen samples were incubated in pollen germination media for a period of 90 minutes. Percent germination of pollen observed are 82.65% in 2.5% sucrose, 51.43% in 3% sucrose and 59.5% in 4% sucrose in 'Hirahalli Dwarf' . In the case of tall cultivar 'Sumangala', it was 47.03% in 2.5% sucrose, 13% in 3 % sucrose and 11.22% in 4% sucrose. The desiccated pollen samples were incubated at three different temperatures (room temperature: 28 to 30ÚC, incubator: 30ÚC, 32ÚC) for a period of 90 minutes to optimize temperature for pollen germination. Maximum pollen germination (75 %) was observed when pollen was incubated at room temperature (28 to 30ÚC).

Pollen germination medium containing 2.5% sucrose, that resulted in maximum germination, was selected as standard for cryopreservation studies also. The desiccated pollen samples were cryopreserved and germination as well as pollen tube length measured. In cryopreserved pollen, germination commenced after 90 minutes whereas in desiccated pollen, it was after 60 minutes. In 'Hirehalli Dwarf', the germination percentages observed were 91% in fresh pollen, 72.2% in dried pollen and 70.1% in cryopreserved pollen whereas in 'Sumangala', it was 72.06 %, 47.03 % and 63.98 % in fresh, dried and cryopreserved pollen respectively. In hybrids (Hirehalli dwarf x Sumangala), germination percentage was found to be 78.3% in fresh, 66.2 % in dried and 61.43 % in cryopreserved pollen.

Viability of cryopreserved arecanut pollen from Hirehalli dwarf for different durations was studied. Germination percentage was found to be 83.4%, 73.8%, 48%, 45% and 42% in fresh, oven dried, cryostored pollen for 24 hours, one month and two months respectively.

Production of normal nut set with the use of cryopreserved pollen indicated its fertility. The study concludes that pollen cryopreservation is feasible in arecanut and can be utilized in large scale hybridization programmes and also for germplasm conservation. Nut set studies conducted at CPCRI showed that normal nut set was observed with two years cryostored pollen from Hirehalli dwarf palms.

5.2 Molecular Markers

Arecanut germplasm have been characterized and evaluated based on morphological and yield parameters (Ananda *et al.,* 2000; Rajesh, 2007). However, the genetic diversity information provided by morphological characters is limited and these parameters can be influenced by environmental, genetic and physiological factors. DNA-based molecular markers are an important tool for evaluating levels and patterns of genetic diversity and have been utilized in a range of plant species and are available in unlimited numbers.

Limited work has been conducted in arecanut for characterization of germplasm. Ananda and Rajesh (2002) utilized morphological and yield parameters and protein profiles for characterization of arecanut germplasm. Cluster analysis, based on protein profiles, did not show any association between geographic and genetic affinities (Rajesh, 2007), highlighting the limitations in using biochemical markers in arecanut. Bagindo (2011) used RAPD for genetic diversity analysis of 30 *Areca catechu* individuals collected in Indonesia (Papua, Sulawesi and Sumatra). Sets of SSR markers were developed by Hu *et al.*, (2009) and Zhan *et al.*, (2012), while Ren and Tang (2010) optimized protocol for ISSR technique in arecanut, but these markers have not been used for detailed genetic diversity analysis of arecanut germplasm.

Recently, genetic relationship among 60 arecanut germplasm, consisting of both indigenous and exotic accessions, were assessed using 14 polymorphic RAPD primers (Bharath *et al.*, 2015). The average polymorphism was 6.64 markers per primer. The PIC values among the 14 primers ranged from 0.19 to 0.49. Similarity values among the accessions ranged between 0.68 and 0.93. Cluster analysis revealed two major clusters. The Indian collections *Konkan I, Konkan II* and *Maidhan* formed a separate cluster. Collections from Indonesia, Sri Lanka, Vietnam, Fiji, Solomon Islands, Singapore China and some Indian collections (Andaman and Nicobar Islands and North East germplasm collections) formed a second cluster. The clustering pattern was, in general, in accordance with the geographical origin of the collections. The results obtained from this study are crucial for developing effective management strategies for genetic improvement of arecanut. The results of the study thus provides the first basic information on the genetic relationship amongst arecanut accessions from diverse geographical regions and can form the basis of advanced studies on germplasm characterization and conservation, breeding programs and selection of possible parents to generate mapping populations of arecanut accessions.

Molecular markers have been successfully applied for the confirmation of fidelity of tissue cultured plantlets. Random amplified polymorphic DNA (RAPD) markers were used to evaluate clonal fidelity of plantlets derived through direct somatic embryogenesis from two mother palms (H and G) of Yellow Leaf Disease (YLD) resistant arecanut (Karun *et al.*, 2008). Pair wise genetic similarities were generated by Jaccard's coefficient using the RAPD banding pattern between each mother palm and its (eight plantlets /palm) progenies. Mother palm H and its progenies were showing maximum similarity (99 %) where as, the mother palm G and its progenies were showed 98 % similarity. The low level of variability shown by plantlets of direct somatic embryogenesis can be exploited for the large-scale multiplication of elite arecanut palms.

Table 2. Summary of the research done on arecanut tissue culture

Explants	Basal Medium	Response	Reference
Zygotic Embryo	Murashige and Skoog	Adventitious shoot formation	Mathew and Philip (2000)
Zygotic embryo	Eeuwens Y3	Varied with varieties – Sreemangala and South Kanara Local gave 93 % germination	Karun *et al.* (2002)
Immature leaf (Seedling)	Murashige and Skoog with picloram and addition of BAP	Plantlet regeneration through somatic embryogenesis	Karun *et al.* (2004)
Immature Leaf – (adult palm)	Murashige and Skoog with picloram and addition of BAP	Plantlet regeneration through somatic embryogenesis	Karun *et al.* (2004)
Inflorescence	Murashige and Skoog with picloram and addition of BAP	Plantlet regeneration through somatic embryogenesis	Karun *et al.* (2004)
Root	Murashige and Skoog with picloram and addition of BAP	Plantlet regeneration through somatic embryogenesis	Radha *et al.* (2009)
Leaf (Seedling)	Murashige and Skoog with BA (0.2 mg/l and TDZ (0.2 mg/l)	Regeneration of plantlets through organogenesis	Wang *et al.* (2003a)
Root (Seedling)	Murashige and Skoog	Regeneration of plantlets through organogenesis	Wang *et al.* (2003b)
Stem-(Seedling)	Murashige and Skoog	Regeneration of plantlets through organogenesis	Wang *et al.* (2006)

Table 3. VARIEITIES OF ARECANUT RELEASED IN INDIA

Variety	Year of Release	Breeding Method (Introduction, Mutation, hybrid etc. with details)	Parents	Important traits	Institute/ University
Mangala	1972	Introduction, evaluation and selection	VTL-3 (China)	Semi Tall palm with partially drooping crown, earliness in bearing, more number of female flowers/Inflorescence, higher nutset, quicker stabilization, round and medium sized yellow coloured nuts. Average chali/dry kernel yield is 3.00kg/ palm/year	CPCRI, Kasaragod
Sumangala	1985	Introduction, evaluation and selection	VTL-11 (Indonesia)	Tall palm with partially drooping crown, oval to round shaped deep yellow coloured nuts. High recovery of chali (26.50%) from fresh fruits. Average chali/dry kernel yield is 3.28 kg/palm/year	CPCRI, Kasaragod
Sreemangala	1985	Introduction, evaluation and selection	VTL-17 (Singapore)	Tall palm with sturdy stem, partially drooping crown. Round and bold with deep yellow coloured nuts. Average chali/ dry kernel yield is 3.18 kg/palm/year	CPCRI, Kasaragod
Mohitnagar	1991	Introduction, evaluation and selection	VTL-60 (W. Bengal, India)	Tall palm with medium thick stem, partially drooping crown, orange yellow coloured oval to round shaped nuts. Higher level of uniformity in	CPCRI, Kasaragod

				performance. The bunches are well placed and nuts loosely arranged on spikes which help in uniform development. Average chali/dry kernel yield is 3.67 kg/palm/year.	
Cal-17/Samrudhi	1995	Introduction, evaluation and selection	VTL-37(Andaman & Nicobar Islands, India)	Tall palm with longer internodes, partially drooping crown. Elongated bold nuts with orange yellow in colour. Average chali/dry kernel yield is 4.34 kg/palm/year and recommended for Andaman & Nicobar group of Islands.	CPCRI and CARI
SAS-I	1995	Introduction, evaluation and selection	VTL-52 (Sirsi Local, India)	Tall palm with compact canopy, deep orange colour with round and even sized nuts. It is suitable for both tender nut and ripe nut processing. Average chali/ dry kernel yield is 4.60 kg/palm/year and recommended for Sirsi hill zone of Karnataka.	UAS, Dharwad
Swarnamangala	2006	Introduction, evaluation and selection	VTL-12 (Saigon)	Tall palm with medium thick stem and comparatively shorter internodes, partially drooping crown. Nuts are bold and heavier with high recovery of chali (26.40 %). Average chali/dry kernel yield is 3.88 kg/ palm/year.	CPCRI, Kasaragod
VTLAH-I	2006	Hybridization and evaluation	Hirehalli dwarf (VTL-56) and Sumangala	Dwarf in nature. Sturdy stem with super imposed nodes, reduced canopy size,	CPCRI, Kasaragod

			(VTL-11)	well spread leaves, medium sized oval to round shaped nuts and early stabilization and medium yielder. Average chali/dry kernel yield is 2.54 kg/palm/year. Advantages-reduced cost of cultivation in terms of harvesting and spraying.	
VTLAH-2	2006	Hybridization and evaluation	Hirehalli dwarf (VTL-56) and Mohitnagar (VTL-60)	Dwarf in nature. Sturdy stem with super imposed nodes, reduced canopy size, well spread leaves, medium sized oval to round shaped nuts and early stabilization and medium yielder. Average chali/dry kernel yield is 2.54 kg/palm/ year. Advantages-reduced cost of cultivation in terms of harvesting and spraying.	
Kahikuchi	2009	Introduction, evaluation and selection	VTL-64 (Assam, India)	Tall in nature with medium thick stem, longer internodes, regular bearer, consistent in yield, bunches are well placed on the stem, orange colour, bold and round shaped nuts, high recovery (25.16 %) of chali from fresh nuts, comes to bearing by 5th year. Average yield is 3.70 Kg dry kernel/palm/ year under normal conditions.	CPCRI, Kasaragod

Madhuramangala	2013	Introduction, evaluation and selection	VTL-62 (Maharastra, India)	Semi tall type, medium thick stem, regular bearer, medium maturity and bearing by 4th year, synchronized maturity of nuts, orange to yellow colour nuts, oval to round shaped nuts, marble appearance in split kernel, suitable for making both chali and processed tender nut. The average yield under normal conditions is 3.54 Kg Chali/palm/Year and 2.95 Kg Dry tender processed nuts/palm/Year.	CPCRI, Kasaragod
Nalbari	2013	Introduction, evaluation and selection	VTL-75(Assam, India)	The yield performance of this variety is higher than the earlier released varieties.Tall type with medium thick stem, shorter internodes, homogenous population, regular bearer, well placed bunches, yellow coloured round shaped nuts, and belong to medium maturity group, suitable for making chali/dry kernel. The average yield under normal condition is 4.15 Kg Chali/ dry kernel palm/year.	CPCRI, Kasaragod

Genetic resources maintained at various institutes:

Crop/ Species	Name of the centre	Number of accessions maintained	Number registered with NBPGR
Arecanut (*Areca catechu* L.)	CPCRI, RS, Vittal	164	83 accessions
Arecanut (*Areca catechu* L.)	CPCRI, RC, Mohitnagar	71	-

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