Advances in Horticulture Vol. 9 – Plantation and Spice Crops Part 1 (1994). Eds: *K.L. Chadha and P. Rethinam* © 1994 Malhotra Publishing House, New Delhi-110 064, India

In vitro Propagation of Coconut and Oil Palm

M.K. NAIR

1. COCONUT

Crop improvement in coconut is a difficult and time consuming programme due to its very long pre-bearing age and prolonged interval between generations. The low multiplication rate of coconut including seed propagation and lack of reproducible clonal propagation technique are other constraints being faced by the coconut breeders. High heterozygosity and predominantly cross-fertilized nature result in enormous variability in the progenies. It is estimated that the total coconut planting material requirement in India is about 15 million seedlings per annum (Anon., 1988). India was the first country to produce a commercial hybrid between tall and dwarf coconut varieties in 1937, and since then the country has released nine hybrids and two varieties. The production of quality planting material in released varieties/hybrids at present is only about two million per annum. While the breeders have evolved high yielding varieties and hybrids, development of an effective protocol for *in vitro* propagation of planting material is still wanting.

1.1 Tissue Culture

The first attempt to culture immature rachillae explants excised from the leaf axil in the country was made by Kuruvinashetti and Iyer (1980). Rachillae explants from unopened spindle were cultured in Y3 medium (Eeuwens, 1976) supplemented with cytokinins, 6-BAP and 2-ip (2 mg/l each). This resulted in formation of shoot like structures; some of which turned to green and even formed roots. The responding spadix was excised by them without damaging the shoot meristem of the palm. Browning was observed in explants from older spathes, and addition of activated charcoal (5 to 10 g/l) reduced the browning considerably. However, viable plantlets could not be obtained from *in vitro* culturing of floral primordia (Iyer, 1982).

Raju *et al.* (1982) initiated work on tender leaf tissue culture using West Coast Tall seedlings (1-2 years old). Explants from apices, tender leaves, leaf bases and leaf sheath were cultured on Y3 basal medium supplemented with auxins (NAA and 2,4-D) and cytokinins (kinetin, BAP and 2-ip). Profuse callusing on 2,4-D (10 mg/l) and kinetin

(0.5 mg/l) media were observed in explants obtained from tender leaf bases. However, limited callusing was observed in the case of explants from tender leaf lamina. Rooting was also observed profusely in this tender leaf cultures. The authors tried more than two thousand combinations of modified Y3 media. They were able to obtain somatic embryoids in 12 per cent of the explants after 16 weeks. By adjusting Mg : K ratio and hormone levels, and avoiding use of 2,4-D, the time was reduced to three weeks. By this process, upto 48 somatic embryoids from a single leaf explant were obtained (Fig. 1). A tripolar structure with root and shoot poles and an incipient haustorial bulge was obtained in individual embryoids (Fig. 2). Attempts were made subsequently to promote the shoot growth by cutting the root poles and part of haustorium. Raju *et al.* (1984) subsequently succeeded in sprouting the embryoids to obtain normal green shoot with scale leaves and sheath leaf base by reducing the auxins by 75 per cent and increasing the cytokinins to 125 per cent.

Raju *et al.* (1988) successfully developed a method for extraction of tender spindle tissues of the mature palm. Two whorls of leaves outside the spindle were removed and the central column of the spindle was exposed by cutting away the spindle 15 to 20 cm above the shoot meristem. The exposed tissues of the palms were sterilized, the tender leaf tissues were dissected and bulk-inoculated into liquid media initially. Subsequently, these tissues were further sub-cultured as small segments and thus from each palm, they were able to establish upto 2500 cultures.

Tissue culture and embryo culture work was initiated at the Plant Research Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi under an *ad hoc* scheme 'Vegetative Propagation of Coconut Palms through Tissue Culture, by a team of scientists headed by Prof. Sipra Guha-Mukherjee. Though they tried to culture explants from endosperm (semi-solid and solid), apical meristems, leaf and leaf base, male flowers, inflorescence, anthers and rachillae, repeatable response could not be obtained (Neera Bhalla-Sarin and Suman Bagga, 1988). They were also able to get few divisions when viable protoplasts were isolated from young leaves in 0.6 M mannitol, incubating in enzyme mixture of 1.5 per cent cellulase, 1 per cent macerozyme and 0.6 M mannitol at 5.8 pH for 16 hours followed by washing in B-5 + 0.6 M mannitol.

Bhaskaran (1985) initiated work on tissue culture of coconut in 1977 at the Tissue Culture Laboratory of Hindustan Lever Ltd., Bombay. He used immature leaf explants for induction of callus and somatic embryogenesis. Somatic embryoids were reported both from induced callus and also directly from mid-rib region of leaf explants by modifying the culture medium. Further, he also obtained calli from immature leaves of coconut seedlings of five varieties and hybrids in media containing high auxin and low cytokinin. By lowering the auxin level, somatic embryogenesis could be induced in calli. Direct embryogenesis from mid-rib region of leaf explants with the number of embryoids per 1 cm explant varying from 10 to 40 was also observed. The embryoids originated from the phloem cells surrounding the vascular bundle without undergoing differentiation to form a callus. Direct and indirect somatic embryogenesis via callus was also obtained in similar culture conditions. However, the embryos arising from callus only showed well-

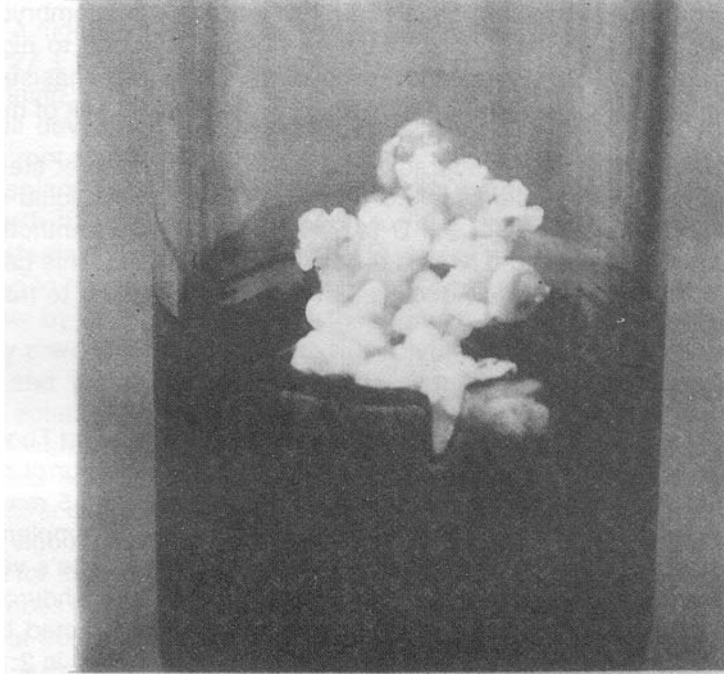

Fig. 1 : Somatic embryoids in coconut.

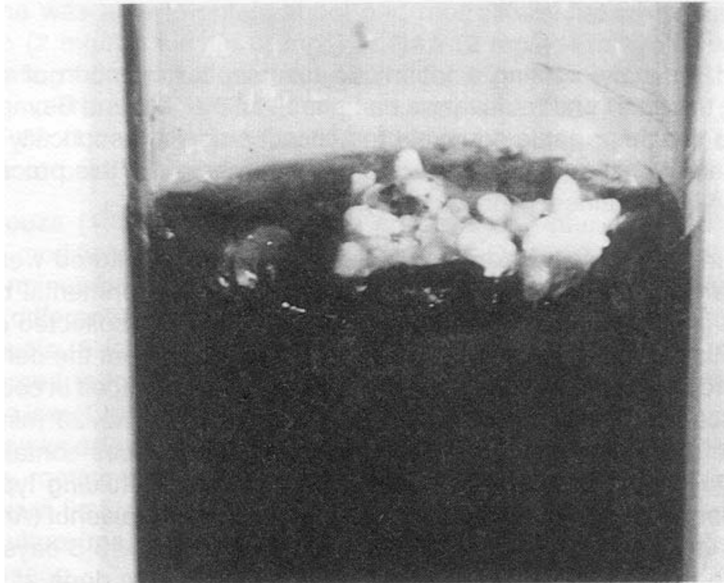

Fig. 2 : Tripolar structure with root and shoot poles.

developed shoot and root apices and haustorium like that in zygotic embryos. Somatic embryos arising via callus germinated as plantlets when transferred to high-cytokinin media (Bhaskaran, 1985). Bhaskaran and Prabhudesai (1986) emphasised the need to produce propagules from a wide range of mother palms to avoid risk of monoculture.

Mascarenhas *et al.* (1988) cultured explants taken from tender stem, leaf and rachillae and tissues of young inflorescence of mature trees on Y3 liquid media. The best response was obtained on Y3 + 2,4-D (4.52×10^2 μM) + Ca panthothenate (2.1 μM) + biotin (4.10 μM) and activated charcoal. Though the stem explants gave globular callus in 10 weeks, no organogenesis occurred even after subculture to fresh medium (Gupta *et al.*, 1984).

1.2 Embryo Culture

The coconut embryo culture was first attempted by Abraham and Thomas (1962) in the Department of Botany, Kerala University. Embryos along with surrounding endosperm was scooped out and embryos measuring 7.5-10 mm long and 5 mm wide were separated and transferred to modified White's medium (White, 1943), supplemented with Nitsch's minor element (Nitsch, 1951), cobalt chloride (25 ppm), White's vitamins and 50 ppm Ca-panthothenate, ferric citrate (10 mg/l), 3-IAA (2 mg/l) casein hdyrolysate (200 mg/l), agar (0.8 per cent) and sucrose (20 g/l). Coconut water extracted from tender nut by sterile syringe was added to autoclaved medium cooled to 40°C in 2:1 ratio. The cultures kept at room temperature (15° to 28°C) produced coleoptile at the blunt end of the embryo; the first leaf appeared in four months and the fully developed plantlet was obtained in 6 months. However, these authors reported only rudimentary growth of root.

The need for standardising a technique for aseptic collection of embryos in coconut, storage, transport and retrieval was emphasised by Raju and Bavappa (1987). They developed a simple portable equipment to collect embryos aseptically in the field in a culture tube and subsequently transferred to fresh media. By this process, 95 per cent germination was obtained (Anon., 1990).

Zygotic embryos excised from immature coconuts and cultured were found to remain viable even after two years of storage without any environmental control and periodic transfers (Anon., 1989). Anitha Karun and Sajini (1994a) collected endosperm embryos along with endosperm cover by means of a cork borer from the dehusked and split open nuts. The extracted embryos from the endosperm was placed in coconut water followed by surface sterilization with 50 per cent chlorine water for 20 min. Embryos were washed with sterile water and directly inoculated to test tubes containing 10-15 ml of Y3 media. the entire operation was done in open using a folding type portable inoculation hood made of plexiglass after surface sterilization with alcohol (Anitha Karun *et al.*, 1993). The embryos collected were kept in an open room for 3-5 days (30-35°C) before transferring to fresh Y3 media. Subsequent transfers were done at an interval of 20-25 days. Initially, the embryos were kept in a dark room (27 ± 1°C) till the germule

emerged in a month's time. The germinated embryos were transferred to a lighted room (2500 lux., 27 ± 2°C) with a photoperiod of 16 hours. The rooting was not successful in the beginning. The media, when supplemented with IBA (5 ppm) and NAA (1 ppm), induced well developed root system. After 12 months of *in vitro* culture, plants with satisfactory root system were transferred into flower pots. As a precautionary measure before transplanting to the pots, the plantlets were treated with bavistin (1 g/l) and thereafter with IBA solution (1000 ppm) for one hour each. The potting mixture used consists of sterile soil, sand and coir dust in the ratio of 1:1:1. The leaves trimmed before transplanting to reduce transpiration. Higher humidity condition was maintained initially at the vicinity by covering it with polythene bags. However, the humidity was reduced gradually by providing perforations to the polythene bag and later lifting the bag during night time and thereafter completely. This process was continued for one month. Hoagland's solution was given to the plantlets once in a month. The establishment of the plantlets were found to be very satisfactory. These authors observed that the vigour of the plantlets was more or less similar and not influenced by the age of the embryos (8 to 11 months old). However, significant correlation was observed between initial root volume and vigour of the plantlets (8 months). Anitha Karun and Sajini (1994b) developed a technique for storing coconut embryos for two months in sterile water. They observed that the chances of contamination by using sterile water is minimum compared to nutrient media. About 80 per cent of the embryos stored in sterile water for two months could be cultured successfully in nutrient media subsequently.

Guha-Mukherjee (1984) raised calluss from various explants such as young (8 to 10 month old) zygotic embryos on B5 medium with IAA-aspartate and IAA-alanine (2 mg/l). She was able to obtain shoot and root differentiation by transferring calli to B5 + IA-asp (2 mg/l) + kinetin (2 mg/l) or NAA (2 mg/l). Embryoids were obtained on roots originating from callus as well as from section of an embryo. She was also able to obtain complete plantlets on B5 medium supplemented with NAA (0.5 mg/l) + BAP (2 mg/l) + PVP (1.0 mg/l). However, three of the plantlets could not be established in the soil due to poor root growth (Neera Bhalla-Sarin *et al.*, 1986).

D'Souza (1980) cultured mature embryos of Tiptur Tall and West Coast Tall cultivars on modified Y3 media (Eeuwens, 1976). On the basal medium without NAA, embryos germinated to produce coleoptile and roots. On transfer to NAA medium before the onset of differentiation, growth was stunted at lower NAA levels (1 to 3 mg/l) whereas at higher levels (5 to 7.5 mg/l) and low chloride and high N levels, the cotyledonary sheath enlarged to form a callus consisting of 'protocorm' and bud-like structures. On subculture to low (1 to 2 mg/l) NAA medium, callus produced roots with pneumatophores. Embryos developed well in liquid medium rather than semi-solid medium. Without high sugar levels, rooting was found inhibited (D'Souza, 1980, 1982; D'Souza *et al.*, 1988). Seedlings were first transferred to a sand-vermiculite mixture and kept under a plastic hood in a culture room. However, the seedlings could not be established further due to contamination.

At Madurai Kamaraj University, Padmanabhan (1982) isolated embryos from mature nuts of West Coast Tall after surface sterilization with 50 per cent alcohol. The

embryos were cultured in Eeuwens (1976) agar medium consisting of BAP, auxins (NAA) and GA_3 with ascorbic acid (AA) as antioxidant at 85 to 90 per cent RH and 30 ± 2°C in diffused light. Padmanabhan (1982) also tried a new medium YNBG consisting of Y3, basic NAA (1 mg/l), BAP (0.5 mg/l), GA_3 (0.5 mg/l) and AA (100 mg/l), wherein a balanced growth of root and shoot was achieved. These cultured embryos took five to seven weeks to form seedlings, compared to 12 weeks in nature and transplantation to soil (Jagadeesan and Padmanabhan, 1982).

Gupta *et al.* (1984) at National Chemical Laboratory, Pune, showed that neither MS nor Y3 agar media supported growth of coconut embryos. But they were successful in obtaining plantlets in two to three weeks in Y3 liquid medium supplemented with CW (10 per cent), BA (4.4 mg/l) and NAA (0.27 mg/l). They observed that large-sized embryos (7 × 4 mm) were more suitable for getting complete plantlets. After conditioning the cultures at 27°C under 16 hours light regime of 1500 lux followed by darkness for eight hours at 25°C, the plantlets were transferred to polybags containing mixture of soil : sand : compost (3:3:1) and kept at 25°C for four weeks in 16 hour photoperiod. However, the plants did not survive beyond four weeks when transferred to glasshouse.

Mascarenhas *et al.* (1988) were able to raise whole plants from West Coast Tall embryos of 5 to 10 mm size grown on Y3 medium (Eeuwens, 1976) supplemented with auxins, cytokinins and activated charcoal; but their attempts to transplant the seedlings to the field were not successful.

The possibility of long-term conservation of coconut embryos was reported by Bajaj (1984) at Punjab Agricultural University, Ludhiana. Zygotic embryos of 1 to 1.5 cm size were stored for a month, partially dehydrated and then cut into two transverse halves. After pretreatment with 7 per cent DMSO and 7 per cent sugar in MS liquid, the embryos were blotted dry, wrapped in sterile aluminium foils and frozen by gradually lowering into liquid nitrogen for five minutes. This was followed by thawing in warm water (35° to 40°C), washing and culturing on MS medium supplemented with 2,4-D (0.2 mg/l), NAA (0.5 mg/l) and kinetin. The cryopreserved coconut embryos and their segments showed a lag period of four months without any sign of growth.

Coconut embryo culture was also attempted at Tamil Nadu Agricultural University by Kalamani and Sree Rangaswamy (1990). Embryos isolated from mature nuts of Tall × Gangabondam hybrids were cultured on four different media, viz., White's, Nitsch's MS and Y3. High shoot differentiation and leaf formation were observed by frequent subculturing in differential medium. Root production was obtained by transferring the cultures to Y3 medium supplemented with NAA (1.5 mg/l) and charcoal (0.1 per cent).

2. OIL PALM

As in the case of coconut, oil palm (*Elaeis guineensis* Jacq.) is a naturally cross-pollinating species and, therefore, yield variable populations in the progenies. Eight to ten years period is required before assessing the fruit yield and oil content in the

population. The advantages of producing good clonal population of selected high yielding trees in oil palm have been described by Corley *et al.* (1977) and Noiret (1981). In addition to the advantages of growing uniformly high yielding clones for individual trees, clonal propagation will also facilitate to propagate *Dura* and *Pisifera* parents to produce desirable *tenera* combinations. This is particularly important to a country like India which is poised for expansion of oil palm cultivation and availability of quality planting material is the major constraint.

Successful vegetative propagation of oil palm through tissue culture has been reported by Jones (1984), Rabechault and Martin (1976), Paranjothy (1984; 1986), and many others from the United Kingdom, Ivory Coast, France and Malaysia. The first attempt in this country to develop a protocol for clonal multiplication of oil palm from tender leaf explants through somatic embryogenesis was by Thomas and Rao (1985) at Bhabha Atomic Research Centre, Bombay. They excised young leaves from 6-month old *tenera* seedlings. Meristematic portions of the leaf base were cut into explants measuring 10 × 15 mm. After surface sterilization, the explants were cultured on a modified MS basal medium with modified vitamins and supplemented with growth regulators. Best callus response was obtained on MS basal medium supplemented with 50-70 mg/l of 2,4-D. Subculturable callus tissues were produced within 6-8 weeks. These callus tissues were periodically transferred to basal medium with reduced levels of 2,4-D and these cultures were maintained as a continuous source for induction of embryogenesis. The cultures were maintained on an optimal medium containing half strength micro and macro elements of MS medium supplemented with sodium dihydrogen phosphate ($NaH_2PO_4.H_2O$, 170 mg/l), 2-4-D (10 mg/l), 2-ip (0.01 mg/l), casein hydrolysate (1.0 g/l) and activated charcoal (0.5 g/l). On transferring the stock cultures to MS medium containing only 2-ip (0.01 to 0.05 mg/l) followed by transfer to basal medium, somatic embryoids occurred within one week. About 50-60 embryos per culture were obtained which gradually developed into shoots. Thomas and Rao (1985) could obtain embryogenesis in both liquid as well as solid medium. Shoots of 10-12 cm size with 2-4 leaves were isolated individually and maintained in tubes with filter paper bridges containing MS liquid medium supplemented with 1 mg/l NAA and 1 mg/l GA_3 to initiate root growth. Plantlets containing 3-4 leaves with roots were transferred to paper cups with pre-sterilized soil compost. Raju *et al.* (1989) reported successful culturing of explants from tender leaf of three-year old *tenera* seedlings. The tender leaf column of seedlings was dissected along with growing apices and after sterilizing with 70 per cent ethanol, the tender leaf was cut into explants of 5 mm length. For induction of callus, the leaf explant was cultured in a medium containing 2,4-D, incubated at 29 ± °C and given a photoperiodic treatment of 16 h of fluorescent light. The relative humidity was maintained at 60-70 per cent. Callus proliferation appeared at the lower cut end of the explant in media where 2,4-D (20 mg/l) along with BAP (1 mg/l) was used.

Somatic embryoids were obtained from one explant with a few cell layers of thick primary callus when cultured in media in which 2,4-D was gradually replaced with NAA. Development of somatic embryoids were obtained in 8-20 weeks. Small white club shaped structures attained the size of 3-5 mm length within 6-8 weeks of their

appearance. However, Raju *et al.* (1989) excluded details of the culture media and growth regulators used. They also claimed that, on a germination medium, about 50 per cent of embryoids developed shoots, 30 per cent had simultaneously shoot and root development (Fig. 3) and 15 per cent with root development followed by shoot. The plants of the size 5 cm, after development of lateral roots, were transferred to a mixture of sand and vermiculite moistened with Hoagland's solution with 0.01 mg/l IBA in mineral pots kept under high humidity condition. Subsequently, these were transferred to the garden mixture kept under shade (Fig. 4).

Jalaja *et al.* (1994) utilized the sprouts of three different *tenera* cross-combinations for clonal propagation of seed sprouts. Sprouts were excised from the kernal sterilized in a mixture of steritab and 0.05 per cent mercuric chloride. After removing the outer sheath of the sprout, it was kept over a filter paper support in shoot apex medium and at the end of 30 days transferred to shoot development medium (Sreenivasan and Jalaja Sreenivasan, 1985, 1992). Among the three different *tenera* combinations tried D 82 × 110 D shoots inoculated, responded well in shoot development medium. From

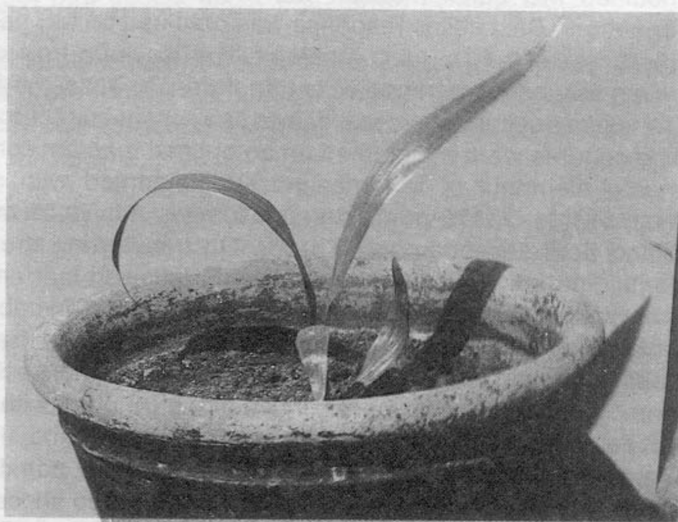

Fig. 4 : Oil palm tissue cultivated seedling transferred to flower pots in garden mixture.

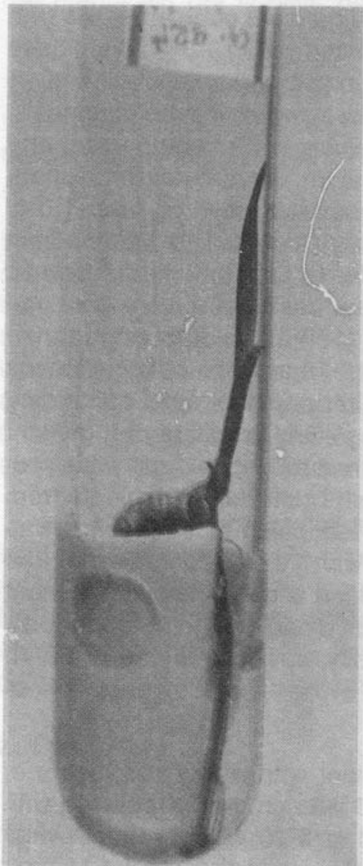

Fig. 3 : Simultaneous root and shoot development in oil palm.

the time of inoculation it took about 150 days to produce 4 side shoots. Root initials started developing after 30-60 days and the root growth was normal with abundant primaries and secondaries. The results obtained by these authors indicated the possibility of inducing the axillary shoots to make available the elite planting material in oil palm.

3. CONCLUSION

The dream of planting coconut clones to meet the ever-increasing demand for elite material is still a distant possibility. The existing gap of more than ten million quality seedlings annually is an indication of the vast potential for the clonal planting materials in coconut and its role in improving the production and productivity. Development of a successful tissue culture technique in coconut is the breakthrough required to bridge the gap between the demand and supply of elite planting materials. Creating, co-ordinating and operating productive tissue culture research among various laboratories in the country is the only possibility to bridge this research gap.

The attempts to standardise a technique for aseptic collections of embryos in coconut, their storage, transport and retrieval have been more rewarding. Techniques are now available for field collection of embryos from distant places, their storage without any contamination upto two months, and successful culturing to develop plantlets and thereby simplifying the procedure for collection of germplasm in coconut.

REFERENCES

Abraham, A. and Thomas, K.J. 1962. A note on the *in vitro* culture of excised coconut embryos. *Indian Cocon. J.*, **15** : 84-88.

Anitha Karun and Sajini, K.K. 1994a. Coconut embryo culture: *In vitro* germination and *ex vitro* establishment. Abst. National Symp. on developments in plant molecular biology and Genetic Engineering, 29-31 December 1993. TNAU, Coimbatore. pp 23.

Anitha Karun and Sajini, K.K. 1994b. Short-term storage of coconut zygotic embryos in sterile water. *Curr. Sci.*, (accepted).

Anitha Karun, Shivashankar, S., Sajini, K.K. and Saji, K.V. 1993. Field collection and *in vitro* germination of coconut embryos—Preliminary observation. *J. Plantn. Crops*, **21** (Suppl.) : 291-94.

Anonymous. 1988. *Proc. of Group Meeting on Strategy for Production of Planting Materials in Coconut, Cashew and Pepper.* Central Plantation Crops Research Institute, Kasaragod, India. (mimeographed, pp. 19).

Anonymous. 1989. Annual Report for 1988. pp. 30-31. Central Plantation Crops Research Institute, Kasaragod, India.

Anonymous. 1990. Annual Report for 1989-90. pp. 40-41. Central Plantation Crops Research Institute, Kasaragod, India.

Bajaj, Y.P.S. 1984. Induction of growth in frozen embryos of coconut and ovules of citrus. *Curr. Sci.*, **53** : 1215-16.

Bhaskaran, S. 1985. Tissue culture technology for higher vegetable oil production. In *Oilseed Prodcution—Constraints and Opportunities* (H.C. Srivastava, S. Bhaskaran, B. Vatsya and K.K.G. Menon, Eds.). Oxford & IBH Publ. Co. Pvt. Ltd., New Delhi. pp. 537-44.

Bhaskaran, S. and Prabhudesai, V.R. 1986. Applications of tissue culture to plantation crops. In *Oilseed Production—Constraints and Opportunities* (H.C. Srivastava, B. Vatsya and K.K.G. Menon, Eds.).

Corley, R.H.V., Barrett, J.N. and Jones, C.H. 1977. Vegetative propagation of oil palm via tissue culture. In *International Developments in Oilpalm* (Earp, D.A. and Newall, W., Eds.). pp. 1-8. Incorporated Society of Planters, Kuala Lumpur.

D'Souza, L. 1980. Hypocotyl budding in cultured coconut embryo. In *Plant Tissue Culture, Genetic Manipulation and Somatic Hybridization of Plant Cells* (Rao, P.S., Heble, M.R. and Chadha, M.S., Eds.). BARC, Bombay. pp. 290-94.

D'Souza, K. 1982. Organogenesis in coconut embryo callus. Proc. 5th Intl. Cong. Plant. Tissue and Cell Culture, Tokyo. pp. 179-80.

D'Souza, L., Paily, J. and Arokiadoss, V. 1988. Coconut embryo and tissue culture: Potentialities, problems and applications. In *Proc. Natl. Sem. Plant Tissue Culture.* 2-4 March, 1983. CPCRI, Kasaragod. pp. 140-45.

Eeuwens, C.J. 1976. Mineral requirements for growth and callus initiation of tissue explants excised from mature *in vitro. Physiol. Plant.*, **36** : 23-28.

Guha-Mukherjee, S. 1984. Vegetative propagation of coconut palm through tissue culture. Project Report of ICAR Scheme 1979-1984. 37 p. (mimeo).

Iyer, R.D. 1982. Embryo and tissue culture for crop improvement, especially of perennials, germplasm conservation and exchange. Relevance to developing countries. In *Proc. COSTED Symp. on Tissue Culture of Economically Impt. Plants* (A.N. Rao, Ed.). Singapore, 1981. pp. 219-30.

Jagadeesan, M. and Padmanabhan, D. 1982. Studies on coconut embryo culture. *PLACROSYM-V* (Abst. 31). CPCRI, Kasaragod, 15-18 December 1982. p. 22.

Jalaja, N.C., Nandhini, M., Sreenivasan, T.V., Nair, M.K. and Naidu, K.M. 1993. Clonal propagation of oil palm (*Elaeis quineensis* Jacq.) hybrid seed sprouts. *J. Plantn. Crops.* (in press).

Jones, L.H. 1984. Propagation of clonal oil palms by tissue culture. *Oil Palms News.*, **17** : 1-18.

Kalamani, A. and Sree Rangaswamy, S.R. 1990. *In vitro* culturing of coconut embryo. *Indian Cocon. J.*, **20** (11) : 9-10.

Kuruvinashetti, M.S. and Iyer, R.D. 1980. Tissue culture studies on coconut palm for its clonal propagation. In *Proc. Natl. Symp. on Tissue Culutre, Genetic Manipulation and Somatic Hybridization of Plant Cells* (P.S. Rao, M.R. Heble and M.S. Chadha, Eds.). BARC, Bombay. pp. 184-91.

Mascarenhas, A.F., Gupta, P.K., Nair, S. and Khuspe, S.S. 1988. Studies on plant tissue culture at the National Chemical Laboratory, Pune. *Proc. Natl. Seminar on Plant Tissue Culture.* 2-4 March 1983, CPCRI, Kasaragod. pp. 198-99.

Neera Bhalla-Sarin, Suman Bagga, Sopory, S.K. and Guha-Mukherjee, S. 1986. Induction and differentiation of callus from embryos of *Cocos nucifera* L. by IAA-congugates. *Plant Cell. Rept.*, **5** : 322-24.

Neera Bhalla-Sarin and Suman Bagga. 1988. *In vitro* culture of embryos and other parts of *Cocos nucifera.* In *Proc. Natl. Semi. on Plant Tissue Culture.* pp. 132-139. Central Plantation Crops Research Institute, Kasaragod. 2-4 March, 1983.

Nitsch, J.P. 1951. Growth and development *in vitro* of excised ovaries. *Amer. J. Bot.*, **38** : 566-77.

Noiret, J.M. 1981. Application of *in vitro* culture to improvement and production of clonal material in the oil palm. *Oleagineux*, **36** : 123-26,

Padmanabhan, D. 1982. Experimental cloning in palms. *S.B. Cl. Newsletter,* **1** (6) : 95-98.

Paranjoty, K. 1984. Oil Palm. In *Handbook of Plant cell Culture* (P.V. Ammirato, D.A. Evans, W.R. Sharp and Y. Yamada, Eds.). Macmillan Press, New York. pp. 591-605.

Paranjoty, K. 1986. Recent developments in cell and tissue culture of oil palm. PORIM Occ. Paper Palm Oil Res. Inst., Malaysia, No. 19, pp. 12.

Rabechault, H. and Martin, J.P. 1976. Multiplication vegetative du plamier a huile *Elaeis quineensis* (Jacq.) a laide de cultures de tissue foliares. *C.R. Acad. Sci. Paris*, **283** : 1735-37.

Raju, C.R., Prakash Kumar, P., Mini Chandramohan and Iyer, R.D. 1982. Attempts at vegetative propagation of coconut palm (*Cocs nucifera* L.) through tissue culture. *Natl. Seminar on Plant Propagation* (Abstr.). Calcutta 27-29 December 1982. pp. 100-101, BCKVV & Soc. Adv. Hort., Kalyani.

Raju, C.R., Prakash Kumar, P., Mini Chandramohan and Iyer, R.D. 1984. Coconut plantlets from leaf tissue cultures. *J. Plantn. Crops*, **12** (1) : 75-78.

Raju, C.R. and Bavappa, K.V.A. 1987. Use of tissue culture for plant quarantine and exchange of germplasm and planting materials in coconut. Paper presented at *Expert Consultation on Use of Tissue Culture in Plant Quarantine for Exchanging Plant Materials.* NBPGR, New Delhi. 26 February-2 March 1987 (mimeo).

Raju, C.R., Shyla, R., Sajini, K.K. and Bavappa, K.V.A. 1988. Attempts at the production of clonal coconut palms. *Natl. Symp. on Coconut Breeding and Management*, KAU, Vellanikkara, Trichur. 23-26 November 1988. Abst. of papers. p. 23.

Raju, C.R., Sajini, K.K., Balachandran, S.M., Saji, K.V., Geetha Maheshan, K., Rajasekharan, P.E., Geetha, L. and Bavappa, K.V.A. 1989. Clonal multiplication of oil palm (*Elaeis quineensis* Jacq.). *J. Plantn. Crops*, **16** (Suppl.) : 17-20.

Sreenivasan, T.V. and Jalaja Sreenivasan, 1985. *In vitro* storage of sugarcane germplasm. *Sugarcane*, No. 1:1-2.

Sreenivasan, T.V. and Jalaja Sreenivasan, 1992. *Indian Sugar Technologies Association. J.* **19** (4) : 61-64.

Thomas, V. and Rao, P.S. 1985. *In vitro* propagation of oil palm (*Elacis guineensis* Jacq.) var. *Tenera* through somatic embryogenesis in leaf-derived callus. *Curr. Sci.*, **54** (4) : 184-85.