ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/242775901

Somatic embryogenesis and plantlet regeneration from leaf and inflorescence explants of arecanut (Area catechu L.)

Article · June 2004

CITATIONS
31

READS
48

4 authors, including:

Anitha Karun
Central Plantation Crops Research Institute
69 PUBLICATIONS **190** CITATIONS

SEE PROFILE

Siril Elenjikkal
University of Kerala
22 PUBLICATIONS **182** CITATIONS

SEE PROFILE

Parthasarathy Va
Bioversity International (CGIAR)
105 PUBLICATIONS **390** CITATIONS

SEE PROFILE

Available from: Parthasarathy Va
Retrieved on: 17 September 2016

Table 4. Important genera (in terms of number of species) of ectomycorrhizae in the world (based on the estimates of Molina *et al.*[1]) and their species number in the present study

Genus (with family in parenthesis)	Species number World	Present study	Predominantly associated with
Cortinarius (Cortinariaceae)	900	4	Conifers
Russula (Russulaceae)	500	13	Oaks
Hygrophorus (Hygrophoraceae)	250	4	Conifers
Inocybe (Cortinariaceae)	210	2	Conifers
Amanita (Amanitaceae)	200	15	Conifers
Lactarius (Russulaceae)	200	9	Both conifers and oaks
Entoloma (Entolomataceae)	160	0	–
Boletus (Bolataceae)	150	12	Oaks
Tricholoma (Tricholomaceae)	150	2	Conifers
Hebeloma (Cortinariaceae)	120	0	–

This study on species diversity of ectomycorrhizal fungi mainly concerns oak and conifer forests of the Western Himalaya. These forest communities are part of a wide altitudinal transect largely dominated by trees having ectomycorrhizal fungi. This feature warrants attention because the transect includes a range of over 15°C mean annual temperature and widely different conditions of topography, soil and precipitation. The diversity values of ectomycorrhizal fungi may not represent the actual picture as not all studies are based on thorough sampling; nevertheless, they give an approximate estimate of ectomycorrhizal diversity which falls within the range described for similar forests elsewhere, thus lending support to certain generalizations relating to ectomycorrhizal diversity at the forest community level.

1. Molina, R., Massicotte, H. and Trappe, J. N., In *Mycorrhizal Functioning: An Integrate Plant Fungal Process* (ed. Allen, M. F.), Chapman & Hall, London, 1992, pp. 357–423.
2. Schmit, J. P., Murphy, J. F. and Mueller, G. M., *Can. J. Bot*., 1999, **77**, 104–1027.
3. Jaenike, J., *TREE*, 1991, **6**, 174–175.
4. Bills, G., Holtzman, F. and Miller, O. K., *Can. J. Bot*., 1986, **64**, 760–768.
5. Cibula, W. and Ovrevo, C., In *Remote Sensing for Resource Inventory Planning and Management* (ed. Greer, J. D.), Aer. Soc. Photog. Remot. Sens., Falls Church, VA, 1988, pp. 268–307.
6. Brunner, I., Brunner, F. and Laursen, G. A., *Can. J. Bot*., 1992, **70**, 1247–1258.
7. Allen, E. B., Allwn, M. F., Helm, D. J., Trappe, J. M., Molina, R. and Rincon, E., *Plant Soil*, 1995, **170**, 47–62.
8. Connell, J. H. and Lowmen, L. D., *Am. Nat*., 1989, **134**, 88–119.
9. Kumar, A., Singer, R. and Lakhanpal, T. N., *The Amanitaceae of India*, Bishen Singh Mahendra Pal Singh, Dehradun, 1990, p. 160.
10. Lakhanpal, T. N., *Mushrooms of India Boletaceae*, *Vol. I*, *Studies in Cryptogamic Botany* (ed. Mukerji, K. G.), APH Publishing Corporation, Delhi, 1996.
11. Lakhanpal, T. N., *Recent Research in Ecology Environment and Pollution Vol. X*: (eds Sati, S. C., Saxena, J. and Dubey, R. C.), 1997, pp. 35–68.
12. Bhatt, R. P., Systematics and ecobiology of some agaric family. Ph D thesis , H.P. University, Simla, 1986.
13. Singh, J. S. and Singh, S. P., *Forests of Himalaya–Structure, Functioning and Impact of Man*, Gyanodaya Prakashan, 1992,
14. Zobel, D. B. and Singh, S. P., *BioScience*, 1997, **47**, 735–745.
15. Adhikari, M. K., *Mushrooms of Nepal* (ed. Durrieu, G.), 1999.
16. Moore, P. D., *Nature*, 2003, **424**, 26–27.
17. Rajaniemi, T. K., Allison, V. J. and Goldberg, D. E., *J. Ecol*., 2003, **91**, 401–416.

Received 14 August 2003; revised accepted 12 February 2004

Somatic embryogenesis and plantlet regeneration from leaf and inflorescence explants of arecanut (*Areca catechu* L.)

Anitha Karun*, E. A. Siril, E. Radha and V. A. Parthasarathy

Biotechnology Section, Central Plantation Crops Research Institute, Kudlu P.O., Kasargod 671 124, India

A protocol for arecanut tissue culture was evolved and observed to be repeatable. It was first standardized with leaf explants excised from one-year-old seedlings and later modified for immature inflorescence sampled from adult palms. The protocol was also tested with different arecanut varieties. 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 the 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 embryos were germinated in MS medium supplemented with cytokinin; 20 μM BA was found to be the best. No variation was noticed for callus initiation, somatic embryogenesis and plantlet development in different varieties except for the period of culturing. To achieve rapid growth and development of germinated somatic embryos, MS liquid medium supplemented with 5 μM BA was used. Plantlets with 2–4 leaves and good root system were veined using sand : soil (5 : 1) potting mixture.

ARECA *catechu* L. is an unbranched, erect, medium-sized monoecious palm growing in hot, humid tropical regions[1]. Apart from its popularity as a masticatory nut, indigenous communities traditionally use it in religious and social functions and it is an ingredient in traditional medicines[1]. Tannin extracted from tender arecanut is considered to be an excellent source of natural dye, tanning agent and ad-

*For correspondence. (e-mail: karun_ani@yahoo.co.uk)

hesive[1]. More than half the arecanut production in the world is from India (53%), followed by Indonesia, China, Bangladesh, Thailand and Malaysia[2]. Seed is the only propagule of arecanut as in the case of many other palm species[1]. This, together with the outbreeding nature of the crop, makes the populations highly heterogeneous and thereby limiting the scope of population improvement programmes in arecanut. Tissue culture seems to be the only vegetative propagation tool applicable to areca palm.

Application of tissue culture technology in clonal multiplication of oil palm[3,4], date palm[5,6] and coconut[7] has been reported, but not in arecanut except for a report on adventitious shoot development from mature embryos[8]. *In vitro* retrieval of arecanut embryos was reported to be successful[9,10]. This communication is a report on repeatable tissue culture protocol in *A. catechu* L.

One-year-old seedlings and 12-year-old adult palms were used for collection of explants. To study the differential response among varieties, seedling explants of three varieties released by the Central Plantation Crops Research Institute (CPCRI), Kasargod[11], viz. Mangala, Sumangala and Mohitnagar were used. As 12-year-old Mohitnagar palms were not readily available, inflorescence explants of a local variety from South Kanara were used.

One-year-old arecanut seedlings were uprooted and thoroughly washed in running tap water. Nine seedlings were destructively sampled for each of the three varieties. After removing the fibrous roots, they were dipped in a 1% systemic fungicide (carbendazim) for 1 h and washed subsequently in distilled water. Each seedling was then swabbed with 70% Et-OH soaked muslin cloth. They were then dipped in 70% Et-OH and flame-sterilized. Three to four outer leaf sheaths were removed and immature leaves were dissected out aseptically. The immature leaves were sliced into 1 mm length and used as explants.

Immature inflorescence was excised by destructive sampling of one adult palm from each variety and grouped into different size classes (< 0.15, 1.6–4.0, 4.1–8.0, 8.1–12.0, 12.1–16.0, and 16.1–20.0 cm) separately and flame-sterilized. Subsequently, inflorescence spathe was removed aseptically. The dissected inflorescence was then sliced into 2 mm length for use as explants.

The basal medium used for callus initiation, somatic embryogenesis and plantlet regeneration was MS medium[12].

Initially five auxin treatments, viz. 2,4-D (68 μM), dicamba (25, 50 μM) and picloram (100, 200 μM) were tried for callus development with seedling explants. In all the treatments, the basal medium was supplemented with 3% sucrose, 0.1% activated charcoal and 0.6% agar. On noticing intensive browning of explants, cultures were transferred to lower concentrations of respective auxins. By the sixteenth week, the auxin level in all the cultures was brought down to 5 μM. Percentage of cultures that produced callus at the end of 16 weeks was used for comparison of treatments.

Based on the results of the aforesaid experiment, only the picloram treatments (T_1, T_2; Table 1) were used for callus development with explants from adult palms.

Somatic embryogenesis was achieved in hormone-free MS medium supplemented with 3% sucrose, 0.2% activated charcoal and 0.6% agar.

Two experiments were conducted to find out the effect of cytokinin on germination of somatic embryos in the case of seedling explants. In the first experiment, somatic embryos were cultured in MS medium supplemented with various levels of BA (0, 5, 10, 20, 30 and 40 μM). Based on maximum percentage germination of somatic embryos in the first experiment, alternative cytokinins, viz. BA (N^6 benzyladenine), kinetin, 2-iP (6-*g*-*g*-dimethylallylamino purine) and TDZ (thidiazuron (1-phenyl-3-(1-2-3-thiadiazol-5-YL) urea)) were tested for a fixed concentration of 20 μM in the second experiment. Besides cytokinin, 3% sucrose, 0.1% activated charcoal and 0.6% agar were also added to the basal medium to obtain germination of somatic embryos.

As BA was found to be the best cytokinin for germination of somatic embryos, different levels of BA (0, 5, 10, 20, 30 and 40 μM) only were tried for somatic embryos derived from inflorescence explants.

Germination of somatic embryos was observed by simultaneous development of shoot and roots. Further transfer of embryos to a filter paper placed on half strength MS liquid media containing 5 μM BA and 3% sucrose, resulted in a continuous growth of germinating em-

Table 1. Changes in quantity of auxin (μM) supplemented with basal medium at different periods of culturing

Auxin	Treatment code	Quantity (μM) supplemented				
		Initial	1st week	3rd week	6th week	16th week
Picloram (4-amino-3,5,6-trichloro picolinic acid)	T_1	200	100	50	25	5
	T_2	100	100	50	25	5
Dicamba (3,6-dichloro-*o*-anisic acid)	T_3	50	50	25	12.5	5
	T_4	25	25	12.5	12.5	5
2,4-D (2,4-dichloro-phenoxyacetic acid)	T_5	68	34	17	8.5	5

bryos. After 6 weeks of culture in this medium the plants were fully developed and produced 2–4 expanded leaves. These plants were used for acclimatization trials. Sand : soil (5 : 1) in plastic pots was used as potting mixture. Plants were initially covered with polyethylene bags and incubated in culture room conditions. Hardening procedure was followed according to the CPCRI coconut embryo culture protocol[13]. Plants watered with MS macro solution once a week, were successfully established. Fully hardened plants were moved to a shaded net-house.

Initially the explants were kept in dark. The temperature and RH were 27 ± 1°C and 80% respectively. Once the calli got initiated, cultures were transferred to light (40 $\mu Em^{-2} s^{-1}$) provided by white, cool fluorescent tubes (Philips) with a photoperiod of 16 h light and 8 h dark. Prior to autoclaving the media at 121°C and 108 kPa pressure, the pH was adjusted to 5.7 ± 02 using 1N NaOH/1N HCl.

All experiments were conducted in completely randomized design with three replications; six culture tubes constituted an experimental unit. ANOVA was performed to test the significance of treatment effects: arcsine transformation was performed on percentages wherever necessary. Statistical procedures as in SPSS v10.0 were used for data analysis[14].

Browning of explants was observed within a week of inoculation. Oxidation and phenolic accumulation followed by rapid explant browning has been common in palm tissue culture[15–18]. Oxidation of phenolics in palm tissue explants was high, but this problem, to a certain extent could be overcome by the use of activated charcoal (1 gl^{-1})[15]. Analysis of variance of percentage browning of cultures at the end of the first week revealed significant difference among varieties and also among the auxins tried (Table 2). Variety-by-auxin interaction was also significant. Browning in Mangala was significantly more compared to Sumangala. Among treatments, dicamba 25 μM and 2,4-D showed significantly less browning compared to the rest. Maximum browning was recorded in picloram 200 μM followed by dicamba 50 μM and was significantly higher than picloram 100 μM. Though variety-by-auxin interaction was significant with regard to browning, in all the three varieties, maximum browning was noticed for picloram 200 μM (Table 2). Leaf slices inoculated in medium with higher concentration of picloram showed rapid explant browning and caused complete darkening. Explant browning was low for explants of Mangala and Mohitnagar in 2,4-D added medium. In these cultures, rapid expansion of leaf explant calli was noticed, which filled the whole media surface within a week.

Serial transfer of explants to low auxin media at varying time intervals (as indicated in Table 1) resulted in the production of callus, except in the medium supplemented with 2,4-D. Percentage callus produced in the treatments was subjected to ANOVA. Significant difference was noticed for callus production among varieties as well as treatments. However, the variety-by-treatment interaction was not significant. Among varieties, maximum callus production was in Sumangala (30%), which was significantly higher than in Mangala (15%) and Mohitnagar (10%) which are on par. Differential *in vitro* behaviour of genotypes has been reported earlier in other crops[19]. Among auxin treatments, significantly higher callus production was noticed in T_3 (33%); callus production in the two picloram-treatments was on par (average = 18.5%). Callus formation was absent in 2,4-D treatment. At this point of time, it was noticed that the callus produced in dicamba-supplemented medium was of the jelly-type, pale yellow or brown in colour and failed to sustain *in vitro*. In the subsequent subcultures it was observed that calli produced in dicamba-contained medium were non-embryogenic. Treatment T_1 (picloram 200 μM series) was found to be the most suitable callogenic agent in all the varieties; white compact, proliferating calli were formed from parallel leaf nerves.

The callusing explants in picloram (5 μM)-supplemented MS medium induced further growth of nodular callus and globular somatic embryos. Pre-germination treatment in hormone-free agar gelled MS medium containing charcoal (0.2%) caused rapid proliferation of callus and also promoted conversion and maturation of globular somatic embryos into elongated structures. It was also noticed that callus obtained from treatment T_1, when transferred to hormone-free medium, recorded significantly more number of somatic embryos (6.44 per culture) compared to 4.73 per culture in T_2. Among varieties, significantly more number of embryos (7.77 per culture) was obtained in Sumangala compared to 4.4 per culture in the other two varieties.

Somatic embryos germinated in MS medium supplemented with BA; few meristemoids were also seen in the cultures. Highest percentage germination was noticed in medium supplemented with 20 μM BA (74.02%) followed by the 30 μM BA supplemented medium (59.17%). Germination was low in the absence of cytokinin (3.7%). Though there was no significant difference among treatments with regard to number of meristemoids, maximum number was noticed with 20 μM BA supplemented me-

Table 2. Percentage browning of arecanut leaf explant cultures in different auxin treatments

Treatment code	Mangala	Sumangala	Mohitnagar	Treatment mean
T_1	94.40	94.40	88.80	92.53
T_2	88.86	55.50	94.40	79.59
T_3	94.40	72.20	94.40	87.00
T_4	72.20	55.50	55.50	61.07
T_5	61.06	77.70	44.40	61.05
Variety mean	82.18	71.06	75.50	76.25

CD (5%) for comparison of varieties = 8.47; for treatments = 10.93 and for interaction = 18.93.

dium (4.78); meristemoids were absent in cultures without cytokinin. In every 6-week interval, germinating embryos were harvested and transferred to half-strength MS liquid medium containing 5 μM BA. Initially meristemoids were dark-green structures with a crown of scale leaves that later advanced to normal shoots. In Sumangala variety, on an average 3.3 shoots were harvested from 20 μM BA-added MS medium.

Comparison of four cytokinins (BA, kinetin, 2-ip, TDZ) at fixed concentration of 20 μM revealed that germination of somatic embryos was significantly more with BA (74.02%) compared to kinetin (45.67%). Maximum number of shoots through organogenesis was also obtained with BA. No significant difference among varieties was noticed for germination of somatic embryos in this experiment.

Culturing of germinated embryos with roots and shoot in half-strength MS medium supplemented with 5 μM BA sustained their further growth. In the case of meristemoids, supplementation of IBA (5 mg l^{-1}) and NAA (1 mg l^{-1}) to half-strength MS medium resulted in successful establishment of plantlets.

Six weeks after culturing in half-strength MS medium, plants with 2–4 leaves and sufficient number of roots were potted in sand : soil mixture (5 : 1) and were grown further and later maintained in a shaded net-house.

Cumulative number of cultures with fast-growing calli, shoots, somatic embryos and plantlets obtained during the first year is shown in Table 3. During the initial period (up to 220 days) the production was low, but it increased thereafter. A total of eight batches of production were recorded in the first year; among these, production was more in the three later batches. The response was more with the Sumangala variety.

Arecanut leaves sampled from adult tress was first tried as explants. However, these explants proved to be less responsive *in vitro*. For instance, leaf slices collected from South Kanara Local gave only 5.53 and 2.65% callogenic response on MS medium with picloram 200 and 100 μM respectively. Alternatively, inflorescence tissues were selected as suitable explants for initiation of cultures.

As in the case of seedling explant, browning was noticed within the first week of inoculation in picloram-supplemented MS medium. Intensity of browning was high in 200 μM picloram-added medium than 100 μM added medium. Subsequently, the auxin level was reduced as described for T_1 and T_2 in Table 1. Nodular callus was observed from the whole surface of the explant (Figure 1 *a*) when cultured in a medium with 25 μM picloram. Subsequently, the cultures were transferred to a medium with 5 μM picloram. Original explants however, turned fully browned by this time. Transfer of explants bearing callus to hormone-free MS medium gave a rapidly proliferating callus line and also promoted somatic embryo development. Somatic embryos initially appeared as globular, smooth, white structures (Figure 1 *b*); they later advanced to elongated structures with a nodal base and tapering anterior end (Figure 1 *c*). They were easily detachable from the callus mass.

ANOVA of callus initiation (at the end of 16 weeks) revealed that the two levels of picloram tried were on par. However, callus initiation was significantly different among varieties. It was also influenced by the size of inflorescence. Interaction between the size of inflorescence and levels of picloram was also significant.

Inflorescence slices cultured in 100 μM picloram-added media gave maximum callogenic response for Mangala (37%) and minimum for Sumangala (21%), while there was significantly higher response with picloram 200 μM (35%). Callus initiation was more or less the same for inflorescence explants of length between 2 and 14 cm. For explants of size less than 1 cm, picloram 100 μM was found to be the best. Response of large inflorescence (more than 16 cm) was significantly inferior compared to the rest. The inflorescence size class ranging from 8 to 12 cm produced maximum callus in all three varieties tested.

Germination of inflorescence-derived somatic embryos was significantly more in medium supplemented with BA (20 μM). Overall, the per cent germination of somatic embryos of the three varieties tried was on par (average = 33.46%). Though interaction between BA levels and varieties was significant, germination was more with BA 20 μM in all the varieties. It is respectively, 66.6, 83.3 and 77.7% in Mangala, Sumangala and SK Local.

The number of meristemoids per culture varied between 0 and 4. BA concentration of 20 and 30 μM produced relatively more number of meristemoids in all the varieties (3.16 and 3.37 respectively).

Germination with simultaneous development of root and shoot resulted in whole plant regeneration (Figure 1 *d*). The transfer of callus to medium supplemented with

Table 3. Total number of cultures with calli, somatic embryos, plantlets at the end of first year of experimentation

Variety	Cultures with fast-growing calli	Shoots separated from calli	Somatic embryos formed	Plantlets developed
Mangala	90	70	284	85
Sumangala	875	110	732	232
Mohitnagar	93	49	105	45

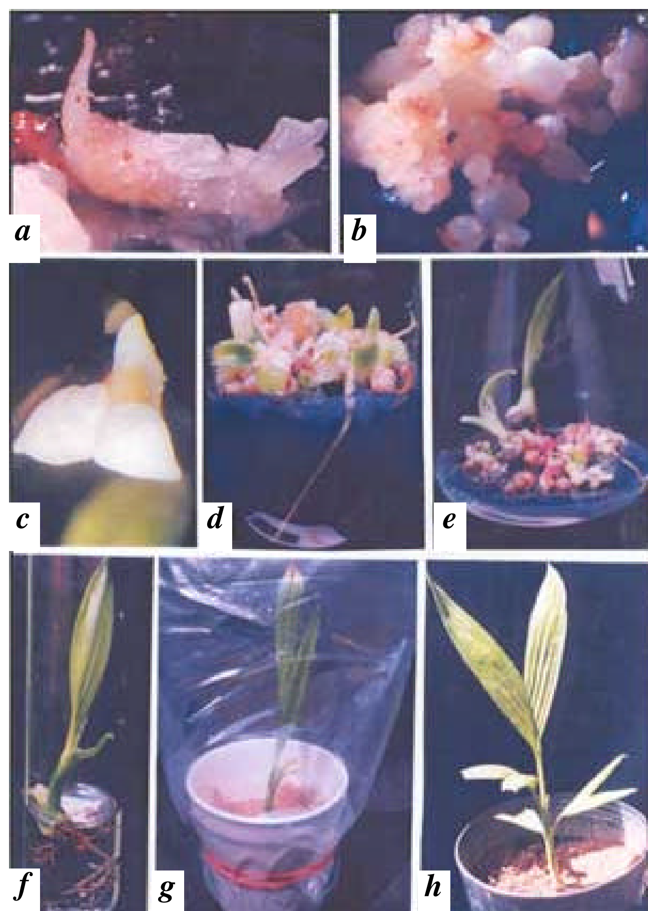

Figure 1. *In vitro* propagation of arecanut through inflorescence explant (var. Sumangala) ***a***, Callus development from inflorescence segment cultured in MS medium containing picloram. ***b***, Embryogenic callus developed in picloram-added medium. ***c***, Fully developed somatic embryos. ***d***, Germination of somatic embryos and meristemoid formation. ***e***, Plantlet development through meristemoids in BA-added medium. ***f***, Somatic embryo-derived plantlet growing in half-strength MS liquid medium containing BA (5 μM). ***g***, Hardening of *in vitro* raised areca plantlet. ***h***, Somatic embryo-derived plant growing in shade net-house.

BA resulted in the development of meristemoid structures identified initially by the dark-green spots that later advanced to scale leaf-covered shoots. However, frequency of meristemoid development was less compared to somatic embryos. In Sumangala, on an average 3.9 shoots were produced in 30 μM BA-supplemented medium. When regenerated shoots (Figure 1 *e*) were separated from the clump and transferred to half-strength MS liquid medium containing 5 μM BA, it resulted in the whole plant development. Plantlets were fully developed after 6 weeks of culture in this medium (Figure 1 *f*). Plantlets with balanced root and shoot system were potted and covered with polythene bags for the first two weeks (Figure 1 *g*). Subsequently, the plantlets were shifted to a shaded net-house and maintained there for further hardening (Figure 1 *h*).

In the present study embryo development is found to be non-synchronous, evident by undifferentiated callus, somatic embryos in different stages, shoot primordial and germinating embryo. This is in contrast with studies on oil palm, where plantlet regeneration was achieved mainly through meristemoids in a medium supplemented with zeatin riboside[3].

Picloram has been used successfully in tissue culture of various plants with no adverse effect on the callus or subsequently regenerated plants[20–23]. Positive response of picloram was reported for callus formation in Pejibaye palm (*Bactris gasipaes* H.B.K.)[24], rattan (*Calamus manan* Miquel.)[25] and *Phoenix canariensis*[26]. Superiority of picloram over other auxins like 2,4-D has been reported earlier[27]. It suggests that there is an effective uptake and mobilization of this growth regulator coupled with rapid metabolization at target sites. BA-induced somatic embryo germination and shoot induction in several palms are available[7]. Further, superior effect of BA over that of other cytokinins on germination has been attributed to the ability of BA to induce endogenous production of natural hormones like zeatin[28].

To summarize, plantlet regeneration is possible by culturing tissues from different growth stages of arecanut: leaf explants from the seedlings and inflorescence explants from adult palms seem to be better. The culture medium and hormonal supplements are essentially the same for the two types of explants, but appropriate changes in the respective concentrations are required for satisfactory results. Somatic embryogenesis and plantlet regeneration could be achieved through continuous monitoring of cultures and by gradually reducing, the concentration of hormones while subculturing. The pattern of callus initiation as well as somatic embryogenesis in different varieties was similar, but the time requirement varied. No variation was noticed for the germination of somatic embryos and further growth of plantlets among varieties. The continuous production of embryonic calli from the initial explants and subsequent somatic embryogenesis indicate the potential of the protocol for mass multiplication of elite palms.

1. Bavappa, K. V. A., Nair, M. K. and Premkumar, T., The Areca palm (*Areca catechu* Linn.). Central Plantation Crops Research Institute, Kasargod, 1982.
2. Rethinam, P. and Sivaraman, K., Arecanut (*Areca catechu* L.): present status and future strategies. *Indian J. Arecanut, Spices Med. Plants*, 2000, **3**, 35–50.
3. Karun, A. and Sajini, K. K., Plant regeneration from leaf explant of oil palm. *Curr. Sci.*, 1996, **71**, 922–926.
4. Rajesh, M. K., Radha, E., Anitha Karun and Parthasarathy, V. A., Plant regeneration from embryo-derived callus of oil palm – the effect of exogenous polyamines. *Plant Cell Tiss. Org. Cult.*, 2003, **75**, 41–47.
5. Tisserat, B., Factor involved in the production of plantlets from date palm callus cultures. *Euphytica*, 1982, **31**, 201–214.

6. Vermendi, J. and Navarro, L., Influence of physical condition of nutrient medium and sucrose on somatic embryogenesis of date palm. *Plant Cell Tiss. Org. Cult.*, 1996, **45**, 159–164.
7. Chan, J. L., Saenz, L., Talavera, C., Hornug, R., Robert, M. and Oropeza, C., Regeneration of coconut (*Cocos nucifera* L.) from plumule explants through embryogenesis. *Plant Cell Rep.*, 1995, **2**, 38–41.
8. Mathew, M. and Philip, V. J., *In vitro* adventitious shoot formation from embryos of *Areca catechu* L. *Phytomorphology*, 2000, **50**, 221–227.
9. Anitha Karun, Siril, E. A., Radha, E. and Parthasarathy, V. A., 2002. *In vitro* embryo retrival technique for arecanut (*Areca catechu* L.). Paper presented at PLACROSYM XV (*Sustainability of plantation crops through integrated approaches for crop production and product diversification*), held at Mysore during 10–13 December 2002, Abstr. no. O-19, p. 46.
10. Ganapathi. T. R., Suprasanna, P., Bapat, V. and Rao, P. S., *In vitro* culture of embryos of arecanut (*Areca catechu* L.). *Fruits*, 1996, **52**, 313–316.
11. Ananda, K. S., Improved varieties and promising traditional cultivars of arecanut. Technical Bulletin (ATIC series of Publication), Central Plantation Crops Research Institute, Kasaragod, 2002.
12. Murashige, T. and Skoog, F., A revised medium for the rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, 1962, **15**, 473–497.
13. Karun, A., Sajini, K. K. and Shivasankar, S., Embryo culture of coconut: the CPCRI protocol. *Indian J. Hortic.*, 1999, **56**, 346–353.
14. SPSS 9 (v-10.0). Manuals SPSS Inc, Chicago, 1999.
15. Tisserat, B., Propagation of date palm (*Phoenix dactylifera* L.) *in vitro*. *J. Exp. Bot.*, 1979, **30**, 1275–1283.
16. Blake, J. and Eeuwens, C. J., In *Tissue Culture of Economically Important Plants* (ed. Rao, A. N.), COSTED, Singapore, 1983, pp. 145–148.
17. Nwankwo, B. A. and Krikorian, A. D., Morphogenetic potential of embryo and seedling derived callus of *Elaeis guineensis* Jacq. Var. *pisifera becc. Ann. Bot.*, 1983, **51**, 65–76.
18. Teixeira, J. B., Sondahl, M. R. and Kirby, E. G., Somatic embryogenesis from immature inflorescence of oil palm. *Plant Cell Rep.*, 1994, **13**, 247–250.
19. Mok, M. C. and Mok, D. W. S., Geonotypic responses to auxins in tissue cultures of *Phaseolus*. *Physiol. Plant.*, 1977, **40**, 261–264.
20. Conger, B. V., Hanning, G. E., Gray, D. J. and Mc Daniel, J. K., Direct embryogenesis of mesophyll cell of orchardgrass. *Science*, 1983, **221**, 850–851.
21. Groll, J., Mycock, D. J., Gray, V. M. and Laminiski, S., Secondary somatic embryogenesis of cassava on picloram-supplemented media. *Plant Cell Tiss. Org. Cult.*, 2001, **65**, 201–210.
22. Beyl, C. A. and Sharma, G. C., Picloram-induced somatic embryogenesis in *Gasteria* and *Harwarthia*. *Plant Cell Tiss. Org. Cult.*, 1983, **2**, 123–132.
23. Lin, H. S., Toorn, C. vander, Raemakers, K. J. J. M., Visser, R. G. F., Jeu, de M. J. and Jacobson, E., Development of a plant regeneration system based on friable embryogenic callus in the ornamental *Alstroemeria*. *Plant Cell Rep.*, 2000, **19**, 529–534.
24. Valverde, R., Arias, O. and Thorpe, T. A., Picloram-induced somatic embryogenesis in pejibaye palm (*Bactris gasipacs* H.B.K.). *Plant Cell Tiss. Org. Cult.*, 1987, **10**, 149–156.
25. Goh, D. K. S., Michaux, F. N., Monteuuis, O. and Bon, M. C., Evidence of somatic embryogenesis from root tip explants of rattan *Calamus manan. In vitro Cell Dev. Biol. Plant.*, 1999, **35**, 424–427.
26. Huong, L. T. L., Baicco, M., Hug, B. P., Burno, M., Santilachi, R. and Rosati, P., Somatic embryogenesis of Canary Island date palm. *Plant Cell Tiss. Org. Cult.*, 1999, **56**, 1–7.
27. Fitch, M. M. M. and Moore, P. H., Comparison of 2,4-D and picloram for selection of long-term totipotent green callus cultures of sugarcane. *Plant Cell Tiss. Org. Cult.*, 1990, **20**, 157–163.
28. Zaerr, J. B. and Mapes, M. O., In *Tissue Culture in Forestry* (eds Bonga, J. M. and Durzan, D. J.), Martinus Nijhoff/Dr. W. Junk Publishers, The Hague, Boston, 1982, pp. 231–255.

ACKNOWLEDGEMENTS. We thank the Director, Central Plantation Crops Research Institute, Kasargod for providing facilities. Financial assistance from the Department of Biotechnology, New Delhi is acknowledged.

Received 17 September 2003; revised accepted 10 February 2004

Performance of *Bt* cotton (MECH-162) under Integrated Pest Management in farmers' participatory field trial in Nanded district, Central India

O. M. Bambawale[1,*], Amerika Singh[1], O. P. Sharma[1], B. B. Bhosle[2], R. C. Lavekar[3], A. Dhandapani[1], V. Kanwar[1], R. K. Tanwar[1], K. S. Rathod[2], N. R. Patange[2] and V. M. Pawar[4]

[1]National Centre for Integrated Pest Management, Indian Agricultural Research Institute Campus, Pusa, New Delhi 110 012, India
[2]Cotton Research Station, Marathwada Agricultural University, Degloor Road, Nanded 431 604, India
[3]College of Agriculture, Naigaon 431 709, India
[4]Marathwada Agricultural University, Parbhani 431 402, India

Farmers' participatory field trail was conducted in 33.18 ha representing rainfed cotton-growing region in Nanded district of the central zone, to evaluate the performance of *Bt* cotton hybrid MECH-162 under Integrated Pest Management (IPM), and to compare it with conventional cotton (CC) hybrids/varieties grown with and without IPM. There was significant reduction in bollworm incidence, particularly the American bollworm (*Heliocoverpa armigera*) and pink bollworm (*Pectinophora gossypiella*) and the damage caused by them to the fruiting bodies in *Bt* MECH-162 compared to CC with IPM. In *Bt* MECH-162, 11.5% of the fruiting bodies were damaged compared to 29.4% in CC with IPM. Maximum damage was observed in CC without IPM, where seven sprays of pesticides were made for control of insect pests in comparison to three on *Bt* MECH-162. Population of the sucking pests and two natural enemies monitored was also lower in *Bt* MECH-162 compared to CC. The latter without IPM recorded the lowest population of natural enemies. Seed cotton yield (12.4 q/ha), and net returns (Rs 16231/ha) were highest for *Bt* MECH-162. CC under

*For correspondence. (e-mail: bambawale1@rediffmail.com)