Status of research on coconut embryo culture and acclimatization techniques in Sri Lanka

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Introduction

The preliminary investigations on coconut embryo culture commenced in 1982 to accomplish successful *in vitro* germination and growth of mature zygotic embryos and to establish *in vitro* seedlings in soil. Several locally available cultivars were used for these studies.

Exchange of coconut germplasm is hampered by several constraints including the large size of the nut, lack of dormancy and phytosanitary regulations. These problems could be overcome if *in vitro* techniques are developed to facilitate the exchange of germplasm in the form of excised zygotic embryos.

Therefore, the main objective of embryo culture research is to develop *in vitro* methods for germplasm collecting and exchange. Embryo culture technology can also be applied to screen drought tolerant coconut germplasm and to rescue embryos of non-germinating types like *Dikiri* coconuts (Makapuno type).

This paper reports the results obtained from previous work and current research activities on coconut embryo culture.

Facilities

The Tissue Culture Laboratory of Coconut Research Institute (CRI), Sri Lanka was established in 1983. The laboratory is quite spacious and well planned. It is well equipped and has facilities for preparation and sterilization of culture media, aseptic culturing and incubation of cultures, and acclimatization of *in vitro* raised plantlets. Equipment needed for histology and biochemical analyses are also available.

Equipment for media preparation: Analytical and top-loading balances, glassware, stirrer/hot plate units, pH meters, micropipettes, ovens and autoclaves.

Facilities for in vitro *culture:* One culture room with 2 laminar flow cabinets and one large incubation room with light and temperature control devices.

Acclimatization facilities: A glasshouse with light and humidity control devices and a screenhouse.

Equipment for histology: Ovens, rotary microtome, light microscopes, inverted transmitted-light microscope, florescence microscope and steriomicroscope.

Equipment for biochemical analysis: HPLC, apparatus for SDS-PAGE and spectophotometers.

Embryo culture technique

Embryo culture technique has been applied successfully for locally available varieties including tall (ordinary tall, *Dikiri* and *San Ramon* forms), dwarf (*pumila, eburnea* and *regia* – 3 colour forms), dwarf x tall and tall x tall.

Mature embryos (11–12 months postanthesis) are excised from the kernel and sterilized in 3% calcium hypochlorite for 5 minutes followed by rinsing in several changes of sterile distilled water. The embryos are cultured in glass tubes (30 X 200 mm) containing 10 ml of the liquid growth medium of which pH is adjusted to 6.0 before autoclaving at 121°C for 20 minutes. Tubes were then sealed with cotton wool plugs and incubated in the dark for two months. This is followed by incubation under 16 hr photoperiod of 6000 lux for 5–6 months. The incubation temperature was 30+1°C. The culture medium in each tube is replenished every month.

Under the present culture conditions, 65%–70% of the cultured embryos develop into complete seedlings when cultured in modified Eeuwens Y_3 (Karunaratne and Gamage 1985) liquid medium. The growth of the plantlets was significantly improved when the mineral, growth factor and sucrose content of the Y_3 formulation was doubled.

Poor rooting, spontaneous senescence of *in vitro* grown plants (specially with *Dikiri* coconuts) and a higher contamination rate of cultures during the latter part of *in vitro* development are some of the constraints encountered in embryo culture.

Experiments were carried out to assess the possibility of reducing cost of embryo culture. It was possible to substitute analar grade sucrose and potassium chloride (KCl) in the growth medium with commercial grade sucrose and fertilizer grade KCl without any negative effects on the growth of the plants as shown in Tables 1 and 2 (Fernando 1994). The feasibility of using tap water instead of deionized water and elimination of hormones (2,4-dichlorophenoxy acetic acid and 6-benzylaminopurine) in the medium are being tested at present to reduce the cost further.

Fully developed seedlings (7–8 months after culturing) having good shoot and root systems, are transplanted in small poly propylene bags (20 cm dia x 15 cm height) containing a pre-sterilized potting 1:1 mixture of river sand and coir dust. They are kept completely covered with poly propylene under low light conditions in the screenhouse for two weeks. Then the plants are gradually exposed to screenhouse conditions for about four weeks. Established seedlings are kept in the glasshouse for about three months until they produced new leaves. They are then transferred to bigger polybags (30 cm dia x 30 cm height) containing a potting 2:2:1 mixture of unsterilized top soil, dried cow dung and coir dust. The plants are exposed to direct sunlight for several weeks before they are planted in the field. During acclimatization, liquid nutrients are applied to the plants at two-week intervals. Plants are watered every other day. Under the present conditions, the success rate is about 60–65%. Experiments are in progress to improve this further by *in vitro* hardening (gradual removal of sucrose at the latter stages), using different potting mixtures (river sand only, river sand and compost, river sand, coir dust and dried cow dung), and different humidity levels.

Field evaluation of the embryo cultured plants (varieties tall and dwarf) has been carried out since 1987. No significant difference in growth parameters, floral structure and bearing pattern has been observed (Jayasekara and Premasiri 1987).

Application of embryo culture technique

1. Germplasm collecting and exchange

Two methods have been developed which are described below.

Short-term preservation method. The mature embryos are sterilized and cultured in screw capped vials (2 per vial with cotyledon buried) containing 10 ml of survival medium. The survival medium is agar based (0.8%) and consisting of one-half strength minerals, vitamins and growth factors of modified Eeuwens Y_3 medium (Karunaratne *et al.* 1985), 60 gl^{-1} sucrose and 0.25% activated charcoal. The cultured embryos are incubated in the dark at 30∞C. When transferred to the germination medium (Karunaratne *et al.* 1985), 53%, 40% and 32% germination was observed after 2, 3 and 5 months of storage, respectively (Karunaratne 1988).

Field explanting method. Aseptic explanting and culturing are done in the field inside an inflatable glove box which can be easily taken to the field. Embryos are cultured directly in the germination medium placed in a vessel called the "Sossou flask" which is specially devised for long collection expeditions. The flask holds about five mature coconut embryos and the flask design prevents spilling of liquid medium and thus facilitates transportation. A case measuring 70X45 X15 cm which a traveler can carry by hand can hold 100 flasks (Sossou and Kovoor 1987).

2. *Embryo rescue of Dikiri coconuts*

Dikiri coconuts containing soft, jelly-like endosperm, (similar to makapuno coconuts) do not germinate *in situ*. Embryo culture technique is applied successfully to rescue embryos of *dikiri* coconuts. Some of the *in vitro* raised *Dikiri* plants have been established in the field and their performance is monitored regularly.

3. *In vitro* screening for drought tolerant coconut germplasm

Studies have been conducted to test the feasibility of developing an *in vitro* method to screen large number of plants for water stress tolerance. Water stress conditions were simulated by incorporation of sodium chloride (NaCl) into the culture medium. The concentration of NaCl was progressively increased from 170 mM to 330 mM.

The results of the preliminary investigations indicated a higher rate of survival with putative drought-tolerant cultivars compared to drought-susceptible cultivars (Karunaratne *et al.* 1991). Seedlings which expressed different levels of tolerance to water stress conditions have been planted in drought-prone areas and performance are being monitored regularly.

The investigations on *in vitro* screening for drought tolerance is continued using polyethylene glycol (PEG) as the water stress simulant.

Future directions

1. *Physiological studies of the field-grown plants which were subjected to* in vitro *drought conditions.*

Physiological parameters such as leaf water potential, stomatal resistance, transpiration rate and cuticular wax content of the plants will be measured in order to compare their drought tolerance capacities under *in vitro* and field conditions. The results of this study will be useful in confirming the validity of the *in vitro* screening procedure.

2. *Studies on the development of specific markers for screening drought-tolerant coconut germplasm.*

Attempts will be made to identify and characterize any specific proteins synthesized in response to water stress using plants subjected to water stress under *in vitro* conditions. This could lead to the development of suitable protein markers for screening drought-tolerant coconut germplasm.

3. *Studies on further improvement of soil establishment of* in vitro*-raised seedlings.*

Physiological parameters such as stomatal distribution and cuticular wax content of *in vitro* grown seedlings will be measured. The resulting information would aid in improving the survival rate of plants during acclimatization stage.

4. *Development of a medium-term preservation technique for coconut germplasm using immature zygotic embryos of coconut.*
5. *Cryopreservation Studies*

At present, the conservation of coconut genetic resources is done through field collections. However, a long-term storage would facilitate the safe conservation of coconut germplasm. Therefore, cryopreservation of mature and immature zygotic embryos of coconut will be tried out as a long-term conservation option.

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References

Fernando, S. C. and E. S. Santha. 1994. Report of the Coconut Research Institute for 1984. CRI, Sri Lanka. Pp. 125.

Jayasekara C. and R. D. N. Premasiri. 1994. Report of the Coconut Research Institute for 1994. CRI, Sri Lanka. Pp. 134.

Karunaratne, S. C. Kurukulaarachchi and C. Gamage. 1985. A report on the culture of embryos of dwarf coconut, *Cocos Nucifera* L. var. *nana, in vitro*. COCOS 3:1-8.

Karunaratne, S. 1988. Short-term *in vitro* preservation of coconut seed material: A method to facilitate field collection and transport of coconut germplasm. CORD IV:40-47.

Karunaratne, S., S. Santha, and A. Kovoor. 1991. An *in vitro* assay for drought-tolerant coconut germplasm. Euphytica 53:25-30.

Sossou, J., S. Karunaratne and A. Kovoor. 1987. Collecting palm: *In-vitro* explanting in the field. Plant Genetic Resources Newsletter. (March 1987). Pp 7–18.

Table 1. Effect of commercial grade sucrose (S) and fertilizer grade KCl (K) on embryo germination and plant development

Treatment	Germination %	Plant development %
Control	78.9	54.1
S	83.9	53.1
K	79.8	45.0
K + S	79.8	62.8
Significance	NS	NS
CV (%)	16.2	20.9

Table 2. Effect of commercial grade sucrose (S) and fertilizer grade KCl (K) on *in vitro* plant growth

Treatment	Shoot height (cm)	Root length (cm)	Leaves/ plant	Roots/ plant	Plants with secondary roots (%)
Control	9.6	3.6	1.4	2.4	69.8
S	8.9	3.5	1.4	2.4	79.6
K	9.7	3.1	1.4	2.2	77.0
K + S	9.4	3.2	1.5	2.1	75.2
Significance	NS	NS	NS	NS	NS
CV (%)	21.7	32.3	19.2	15.3	21.1