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FIELD COLLECTION AND *IN VITRO* GERMINATION OF COCONUT EMBRYOS[1]

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ABSTRACT

A method for collection and inoculation of coconut embryos directly in the field was investigated. Embryos collected from 8-11 months old nuts were surface sterilized and cultured in liquid nutrient medium, inside a portable hood made of plexiglass. The cultures were maintained at room temperature for 3 to 5 days after which they were transferred to fresh medium and stored inside the culture room. The extent of contamination at the time of shoot production was very low. These shoots formed a number of lateral roots in Y-3 medium supplemented with NAA and IBA. The study highlights the possibility of employing the field technique for successful collection of coconut germplasm as zygotic embryos and their retrieval *in vitro*.

INTRODUCTION

Large size of the seed, water content and stringent phytosanitary requirements are the major problems in germplasm collection, transportation and storage of coconut seeds. Alternatively , the zygotic embryos alone can be scooped out and germinated *in vitro.* Many attempts in the direction have been reported in this past (Cutter and Wilson, 1954; Abraham and Thomas, 1962; and Ventura *et al.*, 1966). *In vitro* culture of 'Makapuno' embryos was studied in detail by De Guzman (1970), Balaga and De Guzman (1971), and Del Rosario and De Guzman (1976; 1981). Karunaratne *et al.* (1985) and Assy Bah *et al.* (1987;1989) reported the successful transplantation of *in vitro* seedlings to the field. All the aforesaid investigations were on mature embryos of about 11 to 12 months age. However, such a restriction may limit the number of available nuts during germplasm collection. Two experiments are in progress to investigate the feasibility of field collection and *in vitro* culture of embryos from eight months age onwards. Initial results are reported in this paper. A simple procedure for field collection of embryos and direct inoculation in the field conditions is also described.

MATERIALS AND METHODS

Eight, nine and eleven months old seednuts from WCT palms of CPCRI, Kasaragod were used for the study. The first experiment was started in January 1992 which involved 30 embryos of three different age groups. The second experiment was started in June, 1992 in which 60 embryos were used for each group. Eeuwens (1978) Y-3 media supplemented with sucrose 30 g/l and charcoal 1 g/l were used for the first experiment. For the second experiment, the amounts of sucrose and charcoal were raised to 60 g/l and 2.5 g/l respectively. The pH of media was adjusted to 5.8 before autoclaving.

In order to scoop out the embryos, first the nuts were dehusked and split open. By means of a cork borer the embryo along with endosperm cover were scooped out of the split nut. Such endosperm 'plugs' were cut open and the embryos extracted by using a knife. These embryos were placed in coconut water till the time of surface sterilization. The entire operation was done in the open air on the folding table where the portable inoculation hood (made of plexiglass) was temporarily fixed (Fig. 1). The hood was wiped thoroughly with absolute alcohol to avoid contamination. The embryos were surface - sterilized with 50% freshly prepared chlorine water for 20 minutes. After sterilization, the embryos were washed thoroughly with sterile water 3-4 times. The embryos were then directly inoculated into test tubes/screw cap bottles containing 10-15 ml of culture media.

The embryos thus collected were kept in an open room for 3 to 5 days (temp. 30 to 32°C) before transferring to fresh Y-3 media. Thereafter, periodical transferring was done after every

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21-25 days. Initially, the embryos were kept in a dark room (temp. = 27±2°C) till the gemmule emerged. The germinated embryos were transferred to a lighted room (temp. =27±2°C) with a photoperiod of 16 hours.

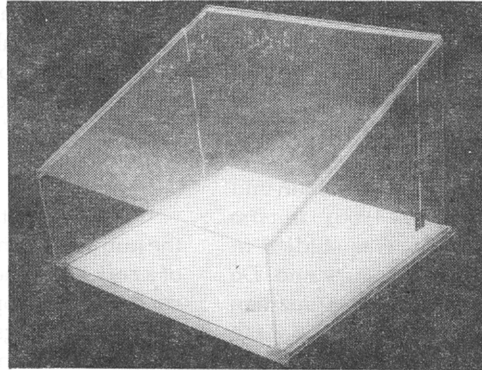
RESULTS AND DISCUSSION

Coconut embryo having no dormant period the elongation of the embryo was observed from first week of culturing, and gemmule started protruding after one month of culturing. The

Table I. Germination and contamination percentages of field collected coconut embryos

Age of the embryos	No. inoculated*		% germination after				Contamination percentage	
			1 month		2 months			
	Jan	Jun	Jan	Jun	Jan	Jun	Jan	Jun
8 months	26	55	26.0	56.4	84.6	58.2	7.69	3.63
9 months	30	59	63.0	62.7	70.0	76.3	3.33	3.39
11 months	30	59	46.0	88.1	70.0	89.8	3.33	3.39

* Embryos damaged while dissecting were discarded.



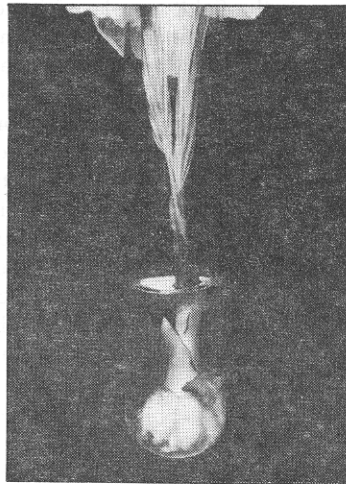


Fig.1 Portable inoculation hood made of plexiglass

Fig.2 Field inoculated embryo cultured plantlet (showing new root initiation from the collar region).

germination percentage and contamination rate in the two experiments are presented in Table I. The lower values of contamination percentages justifies the adequacy of the proposed method of field collection of embryos. The percentage germination in all the cases was satisfactory, except for the eight month-old embryos of second experiment.

After 8 months of inoculation it is found that 30.76% of embryos of 8 months age had not germinated. On the other hand, the percentage of ungerminated embryos in the 9 month and 11 month old groups was very low, being respectively 16.66% and 13.33%. Moreover, the plantlets obtained from 8 month-old category were not as vigorous as those of the other two categories. It seems that certain maturation process/period must elapse before the embryos of this young age can sprout and become seedlings.

Effect of rooting hormones on rhizogenesis

It was observed that for embryos inoculated in the first experiment, the haustorium development was poor; thereby the rooting capacity was not satisfactory. Consequently, the following trials have been conducted to study the effect of growth hormones on rooting.

Embryos that germinated were transferred to Y-3 basal medium containing NAA (1 ppm) and BAP (0.5 ppm). Subsequently, the concentration of NAA was increased to 2 ppm with BAP remaining unchanged, followed by IBA (2 ppm), NAA (2 ppm) where BAP was eliminated. Rhizogenesis in all these trials was found to be unsatisfactory. However, Y-3 medium supplemented with IBA 5 ppm and NAA 1 ppm induced rhizogenesis.

The results indicated the positive action of IBA on rhizogenesis of embryo cultured coconut plantlets. The plantlets cultured on this medium had a well developed root system (Fig. 2) after 15 days of inoculation. There were, on an average 3-6 rootlets emerging from the collar region. Out of 52 treated plantlets, 38 produced fresh thick roots (73%). The performance of these roots after transplanting in the field is yet to be observed.

For a well developed root system, Assy Bah *et al.* (1987) suggested the use of MS basal medium with higher levels of sucrose and NAA (20mg/1-1). De Guzman *et al.* (1971) and Del Rosario and De Guzman (1976) have also emphasized the importance of high levels of sucrose for root initiation in 'Makapuno' embryo cultures. In Sri Lankan dwarf coconut variety, Karunaratne *et al.* (1985) found that when the concentration of sucrose in the medium was increased from 0.09 M to 0.18 M, a substantial increase in size of the haustorium occurred leading to the rapid development of root system within a short period of 6-8 weeks.

Based on the aforesaid observations, the level of sucrose and charcoal in the media used for the second experiment was raised to 60 gms and 2.5 gms per litre respectively. Initial length, width and weight of the embryos, and the weight of embryos before each transfer to fresh media were recorded. The results are summarized in Table II. Eleven-month old embryos, showed significant superiority at all the stages.

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Table II. Size and weight of zygotic embryos inoculated in the second experiment

Age of the embryos	Size at the time of inoculation			Weight of embryos after	
	Length (mm)	Width (mm)	Weight (gm)	1 month (gm)	2 months (gm)
8 months	3.93	3.27	0.052	0.21	0.48
9 months	5.40	3.80	0.090	0.33	0.63
11 months	7.27	4.27	0.120	0.59	0.91
CD (p=0.05)	0.61	0.54	0.050	0.14	0.17

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