Researth Article

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In vitro active conservation of immature zygotic embryos of coconut (*Cocos nucifera* L.) for germplasm collection

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Abstract

A protocol for *in vitro* active conservation of immature zygotic embryos was standardized and observed to be repeatable. Immature embryos collected from 10 and 11 months old nuts of two exotic germplasm *viz.*, Cochin China Tall- (CCNT) and Malayan Yellow Dwarf- (MYD) cultivars of coconut were utilized for the study. Extracted embryos were stored (for 30 days) individually in 5 ml screw capped bottles containing 2 ml of storage medium such as sterile water alone (T1), sterile water with sucrose (7.5 mg/l) (T2), and ¼ strength of Y3 medium containing activated charcoal but without sucrose (T3). The embryos were retrieved on Y3 medium after one month of storage in different treatment media. Significantly higher percentage of germination and healthy plantlets could be obtained from T2 compared to other two treatments. Germination at 60 days after inoculation was significant with type and age factor. Regarding type, germination was more in Dwarf type (93.7%)whereas for CCNT it was 85.6%. Both shoot and root lengths were found to be significantly different among the three storage media compared.

Key words: Coconut (*Cocos nucifera* L.), germplasm collection, embryo culture, storage medium.

Introduction

Coconut zygotic embryos of 11 months age and above can be stored in sterile water for two months (Karun and Sajini, 1994). During germplasm expeditions availability of 11 months and above old nuts is limited making it necessary to collect embryos of lower maturity. The viability of immature embryos in sterile water is however, very low (50-57 %) ; even if germinated, the plantlets are found to be weak as compared to those from mature embryos (92%) (Karun and Sajini, 1994; Karun *et al.,* 1993) which leads to insufficient accession size. Hence, *in vitro* active storage (short- term) of 10 months old embryos in nutrient medium was re-examined in this study (Karun *et al.*,1996).

Materials and Methods

The plant material used in this study consisted of mature (11 months after pollination) and immature (10 months after pollination) embryos of two exotic coconut collections viz., Cochin China Tall (CCNT) and Malayan Yellow Dwarf (MYD) available at CPCRI, Kasaragod. .

Three media namely, sterile water (T1), 7.5 g/l sucrose dissolved in sterile water (T2) and ¼ strength Y3 medium (Eeuwens,1976) supplemented with 1g/l activated charcoal (T3) were compared for *in vitro* active storage of embryos for a period of 30 days. Each treatment consists of 20 embryos and experiment was replicated twice. These cultures were kept in ambient condition for a period of 30 days. The stored embryos were retrieved following the CPCRI protocol of embryo culture (Karun *et al.*, 1998; 2002).

Culture conditions

Initially, the cultures were incubated in a dark room at temperature 27±1°C and 85% RH, till the plumule emerged. The emergence of plumule depends on the variety. After 60 days, the cultures are transferred to an illuminated room (40 $mEm^{-2} s^{-1}$) provided with cool white fluorescent tubes (Phillips), 27±2 °C and RH 85% with a photoperiod of 16 hr.

Transferring the cultures to fresh medium was done once in every 30-45 days. At the time of each transfer, the following characters were recorded: weight gain (g), contamination rate (%), germination rate (%),

pigmentation rate (%), abnormalities such as swelling *(%), shoot length*(cm) and root length (cm). Statistical analysis was done using SPSS v.12.0.

Results and Discussion

Contamination of cultures: At the end of the storage period, cultures were examined for bacterial/ fungal contamination In the case of immature embryos of CCNT 10% cultures were contaminated in sterile water (T1) and sucrose medium (T2).

Retrieval of embryos

After 30 days

The embryos stored in sucrose medium showed better (84%) germination after transferring to retrieval medium. For sterile water, germination was found to be 73 % and for charcoal medium it was close to 63%. Dwarf variety germinated faster(15-20 days) than Tall variety (30-45 days). Significant differences between CCT and MYD embryos were noticed weight gain in retrieval medium, abnormality and contamination at 30 days of inoculation.. Average values of characters studied after 30 days of inoculation are presented in Table 1.

The age factor was found to be significant with regard to weight, abnormality and contamination at 30 days after inoculation. Significant effect of storage medium was noticed with respect to weight and germination after 30 days of inoculation. There was significant interaction between type and age for weight and contamination. Significant type and storage medium interaction effect was noticed for weight after 30 days.

De Guzman *et al.*, (1971), Karunaratnae *et al.*, (1985), Assy Bah *et al.*, (1987) and Rillo and Paloma (1990) reported that embryo development depends on the levels of charcoal and sucrose. The absence of either sucrose or charcoal inhibits germination of embryos and their further growth. Assy- Bah and Engelmann (1993) reported that the mature embryos of dwarf type coconut can be stored for 6 months in MS medium with 2 g/l activated charcoal but without sucrose.

After 30 days of inoculation, no significant difference in germination was observed for type and age, but there is significant difference for storage medium. Age and type factor were found to be significant with abnormality. Abnormality was prominent in Tall type. After one month of growth, the percentage of abnormality noticed in Dwarf type was very less. More abnormality was noticed in mature embryos of CCNT but the percentage of abnormality noticed in charcoal medium was very less. Contaminations showed significant difference with type and age factor. After 30 days of observations, Tall variety showed more contamination than Dwarf, especially the immature embryos were contaminated. It was found that there was significant interaction between type and age for contamination which had no significance with storage medium.

After 30 days of observation, it was found that weight showed significant difference with type, age and storage medium. There were significant interactions between type and age and type and storage medium for weight. The embryos of Tall variety had significant increase in weight than Dwarf. Compared to embryos stored in sterile water and charcoal the embryos stored in sucrose medium had higher weight after 30 days of growth in retrieval medium. Type and age interaction were significant with weight, in which mature embryos of Tall variety showed increase in weight. Likewise, type and storage medium had significant interaction for weight. Tall variety stored in sucrose medium had higher weight.

After 60 days in retrieval medium

Significant differences in germination were noticed (Table 2). Significant effect of storage medium was noticed with respect to weight after 60 days of inoculation.

The germination percentage of embryos of Tall types increased 45-60 days after inoculation. Germination was significant with type and age factor. Dwarf variety germinated promptly. Maximal

Table 1. Average values of characters with regard to factors considered after 30 days of inoculation in retrieval medium.

Characters	Type			Age			Storage medium			
	Tall (CCNT)	Dwarf (MYD)	Avg.	10-month	11-month	Avg.	Sterile water	Sucrose	Charcoal	Avg.
Weight(g)	0.40	0.29	0.35	0.32	0.37	0.35	0.31	0.43	0.30	0.35
Germination(%)	72.92	74.31	73.6	73.3	73.90	73.61	73.13	84.23	63.49	73.61
Swelling (%)	9.40	4.23	6.81	4.40	9.23	6.82	6.32	9.72	4.41	6.82
Abnormality(%)	3.38	0.42	1.90	0.46	3.33	1.90	3.19	1.87	0.63	1.90
Pigmentation(%)	3.43	0.83	2.13	2.17	2.08	2.13	0.00	5.14	1.25	2.13
Contamination(%)	6.20	1.25	3.72	6.62	0.83	3.73	3.33	4.1	3.75	3.73

Table 2. Average values of characters after 60 days of inoculation in retrieval medium

Characters	Type			Age			Storage medium			
	Tall (CCNT)	Dwarf (MYD)	Avg.	10-month	11-month	Avg.	Sterile water	Sucrose	Charcoal	Avg.
Weight (g)	11.27	7.97	9.62	8.55	10.70	9.62	7.90	12.50	8.47	9.62
Germination (%)	85.65	93.70	89.68	84.81	94.54	89.68	93.33	92.01	83.68	89.68
Swelling (%)	2.59	0.45	1.50	1.76	1.25	1.50	1.94	1.94	0.62	1.50
Abnormality (%)	2.64	0.42	1.53	1.39	1.67	1.53	2.57	2.01	0.00	1.53
Pigmentation (%)	2.27	0.00	1.13	2.27	0.00	1.13	2.78	0.00	0.63	1.13
Contamination (%)	7.08	2.08	4.58	7.92	1.25	4.58	3.96	5.42	4.38	4.58

germination was observed in MYD, (93.7%,) and for CCNT it was 85.6%. Mature embryos germinated faster than immature ones. No significant difference in germination was observed among treatments. Significant differences were observed for contamination with respect to age. Immature embryos of both the cultivars showed contamination. There were no significant differences in contamination rate with regard to type and storage medium. Immature embryos of CCNT stored in all the treatment media showed contamination after 60 days of inoculation in the retrieval medium. The embryo weight showed significant difference with respect to cultivation and storage medium. There was also significant interaction between type and age for weight. Regarding age, no significant difference was noticed. For type CCNT, embryos had higher weight. An increase in weight was noticed for all the embryos stored in sucrose medium regardless of type and age. About 5% significance was observed for type and age interaction. CCNT mature embryos grew better.

From the above study, it was observed that abnormalities such as swelling and flaccidity were less in sterile water and were completely arrested in charcoal supplemented medium after 60 days. However, the swelling was more in the embryos stored in sucrose medium. The swelling is due to the absorption of the water from retrieval medium to keep up the osmolarity of the intracellular fluid (Fig.1). Age group studies revealed that after 60 days the immature embryos of both Tall and Dwarf cultivars exhibited more swelling compared to matured embryos. On the other hand, the mature embryos reduced the swelling considerably in the culture at the end of 60 days (Fig.2). Germination percentage of such embryos was 94 %. Karun and Sajini (1994) reported that the germination of immature (8- month old) embryos of WCT after 2 months of storage was negligible; their response being. 20,0, 6.67, 0.00 percent in Y3 medium with sucrose, Y3 medium with charcoal, and sterile water respectively.

In immature embryos, the swelling increased

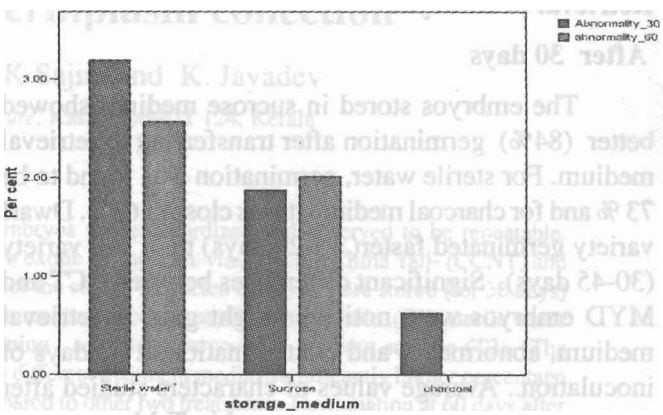

Fig.1. Abnormalities in embryos noticed after 30 and 60 days after inoculation in retrieval medium with respect to various storage media.

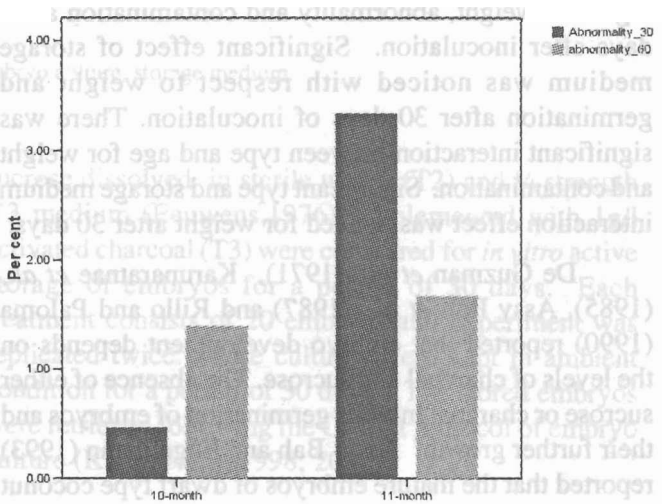

Fig. 2. Abnormalities in embryos noticed in two age groups (mature and immature) after 30 and 60 days

after 60 days in culture (Fig.2). This swelling abnormality did not interfere with germination. The germination percentage of immature embryos after 60 days was 85 (Table 2).

Shoot –Root growth

Protuberance of the plumule started after a week in Dwarf and after 2 weeks in Tall variety. The embryos stored in sucrose medium produced roots faster than in sterile water and charcoal(Table 3).

Table 3. Shoot and root development (%) after 50 days of inoculation

	10 month after fertilization		11 month after fertilization	
	CochinChina	MYD	Cochin China	MYD
Sterile water	0.54 (0.2)	0.35 (0.27)	0.48(0.27)	0.36(.66)
Sucrose	0.77(0.6)	0.43(2.6)	5.4(2.01)	0.50(2.7)
Charcoal	0.53(1.03)	0.48(1.25)	0.39(3.5)	0.34(1.6)

Figures in the parentheses are the values of root development

Root length was significantly different between mature and immature embryos. Both shoot and root lengths were found to be significant among the three storage media compared. Root growth was faster in embryos stored in sucrose medium (Table 3). Shoot length (cm) of plantlets was significantly different between CCNT (0.4291 cm) and MYD (0.3179 cm). It was observed that the plantlets derived from the embryos stored in sucrose medium gave balanced shoot and root growth (Fig.3). The plantlets obtained from sterile water stored embryos produced healthy shoot system with less root systems. Such types of plantlets need to be subjected to rhizogenesis medium (Karun *et al.,* 1993)

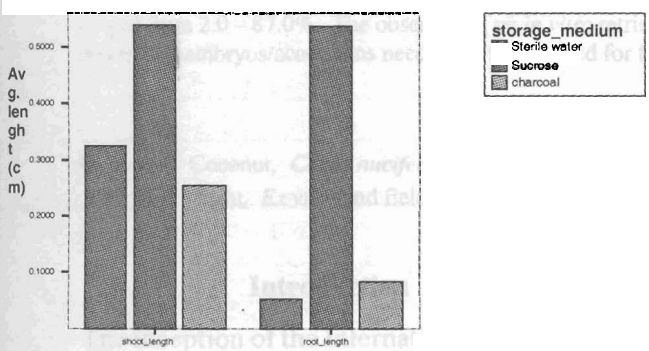

Fig.3. Average shoot length and root length (cm) of embryo cultured plantlets after subjecting them to three storage media.

Assy -Bah and Engelmann (1993) stored coconut embryos in liquid medium containing 2 g/l activated charcoal without sucrose and obtained 77 % germination. They found that increased levels of sucrose in the absence of charcoal affected the viability of embryos. Karun and sajini et *al.,* (1994) reported that none of the immature embroys could germinate after storing them for eight months in sterile water for two months. After 150 days of inoculation in retrieval medium, 62.5 and 82.5 percentage of plantlets could be retrieved from immature embryos of CCNT and MYD respectively in sucrose medium. Plantlets obtained from this treatment (embryos inoculated in sucrose -T2) had uniform root and shoot development as compared to sterile water and charcoal medium. The rooting percentage varied from 15- 37.5 as compared to 2.5 to 23 and 10 to 15 in sterile water and activated charcoal medium respectively. The overall plant health of immature embryos was significantly higher in sucrose medium. Per cent (mean) shoot and root development after 150 days of observation are shown in Table 4.

Table 4. Mean Percentage of plantlets with shoot and roots after 150 days of inoculation

	10 months after fertilization		11 months after fertilization	
	Cochin China	MYD	Cochin China	MYD
Sterile water	70.00 (25.00)	40.00 (2.50)	82.5 (2.50)	42.5 (2.5)
Sucrose	62.5 (22. 50)	82.5 (37.50)	92.5 (37.5)	77.5 (15.00)
Charcoal	55.00 (15.00)	82.5 (15.00)	82.5 (15.00)	50.00 (10.00)

Figures in the parentheses are the values for root

Conclusions

The present study aimed to select the best storage medium for rescuing immature coconut zygotic embryos during exotic germplasm collections. The mature (11 months) and immature zygotic embryos of exotic coconut germplasm of CCNT and MYD were subjected to three different storage media for a duration of 30 days. The germination of embryos and healthy plantlet development with abnormalities were studied. Germination at 60 days after inoculation was significant with cultivar and age factor, 85 per cent of immature embryos having germinated as against 95 of the mature embryos. Regarding cultivar, germination was more in Dwarf type (93.7%); than for CCNT (85.6%). Both shoot and root lengths were found to be significant among the three storage media compared. Immature embryos stored in sucrose exhibited healthy and better shoot (62.5 and 82.5 %) and root growth 22.5 and 37.5 in CCNT and MYD respectively. This is comparable with that of matured embryos stored in sucrose medium.

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