Journal of Plantation Crops 24 (Supplement) : 586-593 (1996)

IN VITRO ACTIVE CONSERVATION OF COCONUT ZYGOTIC EMBRYOS

ANITHA KARUN, K.K. SAJINI and R.D. IYER
Central Plantation Crops Research Institute, Kasaragod 671124, Kerala, India.

ABSTRACT

***In vitro* active conservation of coconut zygotic embryos was found to be possible for 2-4 months. Mature embryos can be stored in sterile water for 4 months. For 2 months storage, sterile coconut water was found to be adequate. Embryos could also be stored for 2 months after Na-alginate encapsulation. The germination of the stored embryos and further development were similar to those not stored.**

INTRODUCTION

In vitro tissue culture technique can be of great value for germplasm collection, storage and multiplication of recalcitrant species, which are high in moisture and are unable to withstand much desiccation. Collection and transport of coconut germplasm in the form of embryo cultures instead of seed nuts has many advantages. A simple procedure for direct field collection of 8- 11 month old coconut embryos and their *in vitro* germination was described by Karun *et. al.* (1993). However, immediate transport of the field collected embryos may not be possible where the collection sites are located in distant places. This necessitates the *in vitro* active conservation (short-term storage) of coconut zygotic embryos.

In vitro active conservation can be achieved by maintenance of cultures in the growing state, usually with growth slowed down by limiting the physical environment or culture medium composition (Withers. 1990). Storing in reduced strength of nutrient medium, low temperature or manipulation of gaseous environment are some of the common procedures employed for this purpose. Response of coconut zygotic embryos stored in the above manner for 2-6 months is reported in this paper.

MATERIALS AND METHODS

The zygotic embryos of 11 months (mature) and 8 months (immature) old West Coast Tall cultivar were subjected to storage for 2-6 months. Extraction of embryos from the nuts and surface sterilization were done as described by Karun *et. al.* (1993). The stored embryos were then transferred to the retrieval medium to test their viability.

Experiment I: A factorial experiment with two types of embryos (mature and immature), three periods of storage (2, 4, and 6 months) and three storage media viz., Eeuwens' Y3 medium without charcoal (S1), Y3 medium without sucrose (S2), and sterile water (S3) was initiated during April 1993. The retrieval medium (i.e., full Y3 medium with 4% sucrose. 2 g/l activated charcoal and 5 g/l agar) was included as control. The pH of all the media were adjusted to 5.7. Five embyros each were inoculated in the respective treatments and the experiment was replicated thrice. The stored embryos were kept in uncontrolled environment (i.e., room temperature (30 2°C): RH 75 10%). On completion of the respective storing periods, the embryos were transferred to the retrieval medium and the cultures were then maintained as described in detail by Karun *et. al.* (1993).

Experiment II. This experiment was started in July 1993 to know the feasibility of storing mature coconut embryos following encapsulation. Extracted embryos after surface sterilization were encapsulated with sodium alginate (3%), $Cacl_2$ 0.1M and washed in sterile water before transferring to a conical flask containing 10 ml of sterile water. Ten embryos were kept together in a conical flask and sealed air tight with parafilm (fig. 1). One set was stored in a refrigerator at 10°C and one set in room temperature for 3 months.

Experiment III. To determine the maximum storing period of mature coconut embryos in sterile water, an experiment was started in November, 1993 in which the viability of stored embryos was assessed periodically after 2, 4, 6 and 8 months of storage.

Experiment IV. Embryos were stored in sterile coconut water in this experiment (started in January, 1994). Sterile coconut water was extracted by using sterile filters (0.22m - Millipore). Extracted mature embryos were surface - sterilized and directly inoculated into 15 ml vials containing 5 ml of sterile nut-water of the same nut. Individual vials were sealed air tight with parafilm and kept in a dark room. A total of 60 embryos were inoculated, and after every 2 months 30 were transferred into retrieval medium to assess their ability to germinate.

Experiment V. In this experiment, mature coconut embryos were inoculated into 1/2 concentration of Y3 medium with 2 g/l charcoal and without sucrose, and in sterile water (as control), and stored at different temperatures, namely 25°, 30°C and room temperature (30° ± 2°C). Twenty embryos were used for each treatment combination and the experiment was replicated thrice.

RESULTS AND DISCUSSION

In vitro active conservation of zygotic embryo of mature coconuts was possible for 2-4 months. The germination of the stored embryos and further developments were similar to those not stored. On transferring to the retrieval medium, the stored embryos germinated within 35-40 days of inoculation, except in experiment IV (coconut water as storage medium), in which it took 65 days. It is observed that embryos that turned completely brown in the storage medium did not germinate later. Germination in case of immature embryos was not satisfactory.

Minimal growth media

Various studies indicated that germination of coconut embryos and their further development depended greatly on the levels of activated charcoal and sucrose (De Guzman *et. al.*, 1971: Karunaratne *et. al.*, 1985; Assy Bah *et. al.*, 1987; and Rillo and Paloma, 1990). The absence of either charcoal or sucrose is thus expected to inhibit the germination of embryos and their further growth. Results of Experiment - I suggest that mature coconut embryos can successfully be stored for two months in the recommended retrieval medium devoid of either charcoal or sucrose. The viability of embryos was not lost even when they were stored in sterile water for two months. The per cent germination after two months of transfer to retrieval medium is comparable with that of control (Table I) and with the previous studies (70-89%) of Karun *et. al.* (1993). Because of severe fungal contamination (from the filter paper bridge), these media were not evaluated for their suitability for storing embryos for 4 months. Germination of embryos after 6 months of storage was very low (Table I).

Assy Bah and Engelmann (1993) found that dwarf coconut embryos can be stored for 6 months in a medium containing 2 g/l activated

charcoal without sucrose, which gave 77% germination. They have also reported that increased levels of sucrose in the absence of charcoal affected the viability of embryos, and germination was negligible in the absence of both charcoal and sucrose. These results indicated that embryo development is promoted by the presence of sucrose and the toxic by products are absorbed by actual charcoal, and thus, favouring further growth. Abnormal swelling of embryos was noticed in the medium SI, which is devoid of charcoal but with sucrose (Fig. 1).

Sterile water as storage medium has many advantages in the collection and exchange

Table I. Percent germination, contamination and browning of stored embryos in minimal growth medium after 2 months of transfer to the retrieval medium.

Experiment no.	Medium of storage	Period of storage (months)	Embryos stored (no.s)	Germination (%)	Contamination (%)	Browning (%)
Expt. I : (Mature embryos)						
	Y3 without charcoal (S1)	2	15	66.6	6.6	6.7
		6	15	6.6	56.3	40.0
	Y3 without sucrose (S2)	2	15	66.7	0.0	0.0
		6	15	13.3	0.0	6.6
	Sterile water (S3)	2	15	80.0	0.0	53.3
		6	15	13.3	0.0	73.3
	Control (S4)	0	15	66.7	3.3	0.0
Expt. II : (Immature embryos)						
	S1	2	15	20.0	0.0	20.0
	S2	2	15	6.7	0.0	0.0
	S3	2	15	0.0	0.0	46.7
	S4	0	15	20.0	0.0	0.0
Expt. : III Sterile Water		2	50	72.0	0.0	40.0
		4	50	62.0	2.0	40.0
		6	50	6.0	0.0	70.0
Expt. IV : Sterile Coconut water						
		2	30	66.6	23.3	13.3
		4	30	6.7	6.7	86.7

* A part of this experiment is reported by Karun and Sajini (1994).

of coconut germplasm, since the chances of contamination by using nutrient medium are high. Moreover, it is observed that embryos stored in sterile water germinated uniformly on transferring to retrieval medium (Fig. 2). Use of sterile water as a storage medium for coconut embryos was first reported by Karun and Sajini (1994).

The per cent germination of immature embryos on transfer to retrieval medium was negligible (Table I). In the control too, their performance was not satisfactory, when compared to earlier results obtained under controlled condition (58-84%) by Karun *et al.* (1993). None of the immature embryos germinated following the storage for 4 and 6 months.

In Experiment III, embryos could be stored upto 4 months in sterile water. In order to control the contamination, the embryos were kept without any filter paper support. The per cent germination of embryos stored for 2 and 4 months was found comparable and satisfactory (Table I). However, the germination of embryos stored for 6 months embryos was very low and intensive browning was noticed.

Results of Experiment IV suggest that for germplasm collection and transportation, sterile coconut water can also be used as a storage medium. Beyond 2 months, the viability of mature embryos declined as can be seen in Table I. It was noticed that embryos stored in nut water took a long time for germination in the retrieval medium. The elongation of the embryo itself was very limited.

Encapsulated embryos. Encapsulation techniques are now widely used in the "synthetic seeds" technology by coating somatic embryos /shoot meristems in sodium alginate beads. Encapsulation will provide the planting material with increased resistance to dehydration and low temperature (Englemann, 1991). While six out of 10 encapsulated coconut embryos stored at room temperature (Fig. 4) for two months had germinated when transferred to retrieval medium, none of the embryos stored in the low temperature (10° C) germinated. This is indicative of the deleterious effect of low temperature on the viability of coconut embryos.

The germination and further development of encapsulated embryos were similar to that of normal unstored embryos. In this case also, the sprout gemmule emerged after 30-35 days of inoculation in the retrieval medium. The plantlets obtained from encapsuled embryos were transferred to pots after 6 months of *ivitro* culture. Sandalwood somatic embryos and mulberry buds were reported to be successfully stored for 45 days at 4°C after encapsulation

Table II. Percent germination, contamination and browning of coconut embryos stored for 3 months (Experiment - V) after 2 months of transfer into the retrieval medium

Medium of storage	Temperature (°C)	Germination (%)	Contamination (%)	Browning (%)
1/2 Y3 + 2 g/l charcoal	25	40.0	23.3	0.0
	30	23.3	23.3	0.0
	Room Temp.	6.7	30.0	0.0
Sterile water	25	0.0	0.0	50.0
	30	23.3	0.0	76.7
	Room Temp.	30.0	6.7	63.3

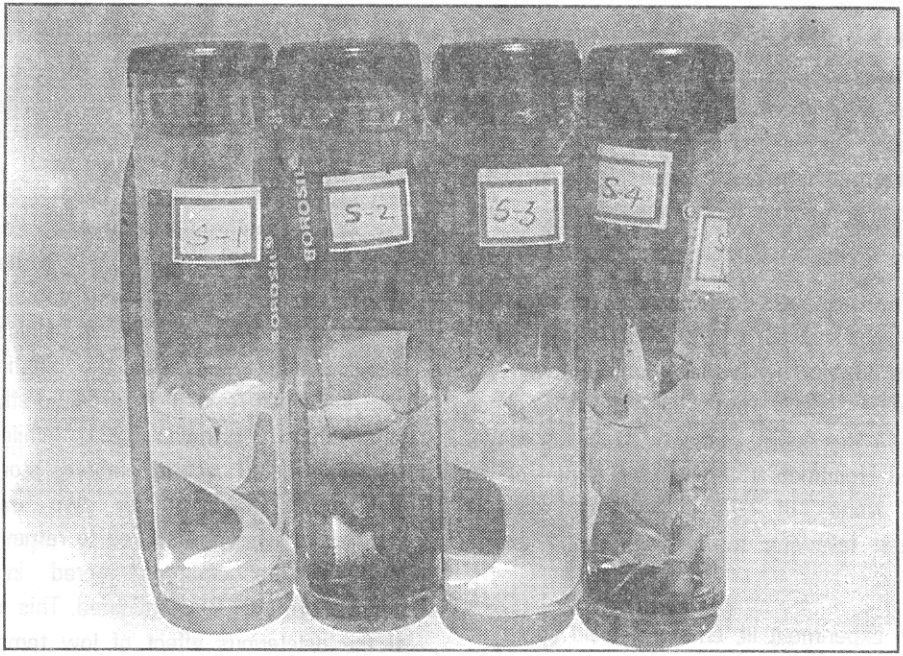

Fig 1. Coconut embryos stored for two months in minimal growth medium. S1 - Eeuwens' Y3 medium without charcoal : S2 - Eeuwens' Y3 medium without sucrose; S3 - Sterile water (note the slight browning at shoot-root apex) ; S4 - Control (Retrieval medium)

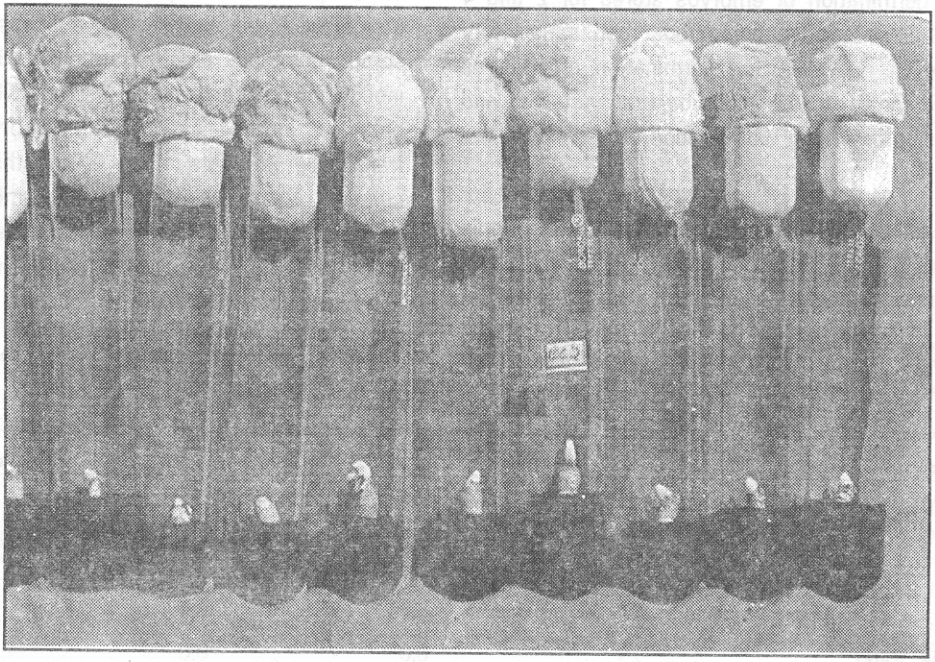

Fig 2. Uniform germination of the embryos stored for two months in sterile water after transferring to retrieval medium (note the gemmule emergence).

Fig 3. Abnormal swelling noticed in the retrieval medium for embryos stored in Eeuiwens' Y3 without charcoal for two months.

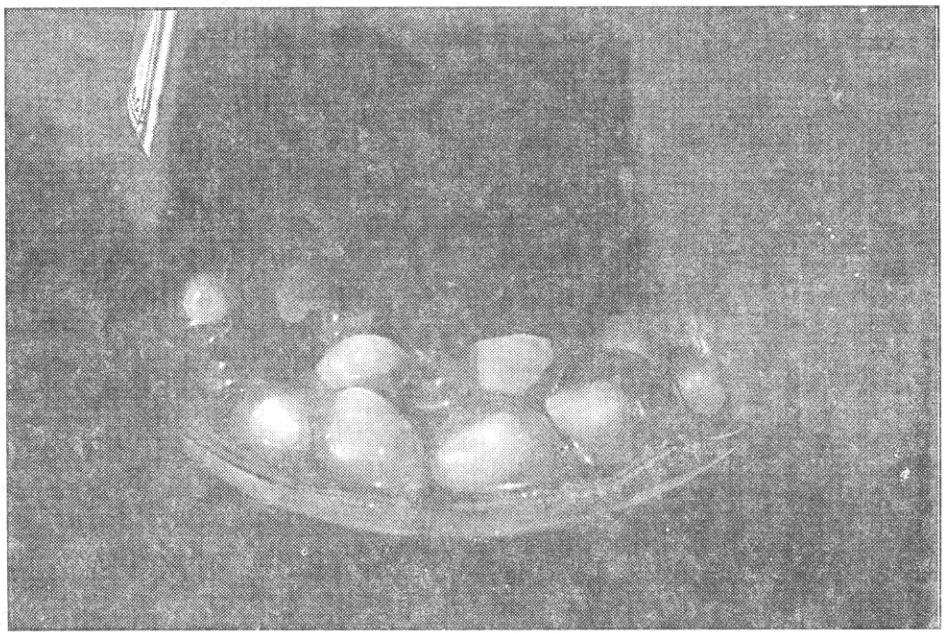

Fig 4. Encapsulated coconut zygotic embryos in algenic salt of Sodium (3%) and stored at room temperature.

(Bapat *et. al.,* 1987; Bapat and Rao, 1988). Ours is the first report on *in vitro* active conservation of encapsulated mature zygotic embryos.

Temperature and low nutrient media. Tropical plant species are generally cold sensitive and low temperature often induce physiological damage and these increase with the degrees of chilling (Lyons, 1973 ; Graham and Patterson, 1982). The storage temperature depends on the cold sensitivity of the crops. For example oil palm plantlets and somatic embryos are not able to resist relatively short exposures to temperatures lower than 18°C (Corbineau *et al* 1990). In a preliminary study, mature and immature coconut embryos stored at 10°C for one month did not germinate on transfer to retrieval medium. Hence, the low temperature on transfer to retrieval medium. Hence, the low temperature for storing the coconut embryos was fixed as 25° C in Experiment - V. After storing the embryos for 3 months, it was observed that embryos stored in sterile water showed more browning, and the embryo elongation and subsequent growth and development were hastened in embryos stored in half concentration of Y3 medium. Per cent germination, contamination and browning observed in this experiment are given in Table II.

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