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Efficacy of CPCRI protocol of Coconut embryo culture in germplasm expedition

Anitha Karun, K. K. Sajini, E. Radha and V.A Parthasarathy

Central Plantation Crops Research Institute, Kasaragod-671 124 Kerala

Abstract

The CPCRI protocol of coconut zygotic embryo culture was utilized for five germplasm expeditions during the period 1997-2001. A total of 4182 embryos of 45 accessions were collected from 8 countries *viz.*, Mauritius, Madagascar, Seychelles, Maldives, Comoros, Reunion, Sri Lanka and Bangladesh. The per cent retrieval of embryos varied among the location and among accessions. A major cause of differential germination was the contamination of cultures, which could be partially attributed to the personal skill and the conditions of the site at the time of collection. Treatment of cultures with tetracycline (2 ppm) be effective in treatments of cultures with mild bacterial contamination. The per cent germination varied between 54 (Sri Lanka) to 82.2 % (Bangladesh) between expeditions. In earlier expeditions when seed nuts were brought, the germination ranged from 2.0 – 87.0%. The observation on *in vitro* retrieval of embryos and their *ex vitro* establishment suggest that, about 300 to 400 embryos/accessions needed to be collected for field establishment of 100 plants in a gene bank.

Key words: Coconut, *Cocos nucifera* L ., Coconut germplasm, embryo culture, field collection , *In vitro* retrieval, ICG-SA Kidu, *Ex vitro* and field establishment

Introduction

The inception of the International Coconut Gene Bank – South Asia (ICG-SA) at the Research Centre of CPCRI at Kidu necessitates the collection and conservation of large number of exotic germplasm. The objectives of the gene bank also included sharing of germplasm between countries. For programmes of this kind, transportation of germplasm in the form of excised embryos is considered to be more safe, economical and easy. Any protocol to meet this requirement should retrieve embryos of different types of accessions. Further, the method of collection of embryos should be simple as only minimum facilities can be expected at collection sites. Besides, it should be easy to practice so that no restriction may arise due to non-availability of trained personnel for germplasm collection. How far the CPCRI protocol of zygotic embryo culture meets the aforesaid requirements is examined in this paper based on data gathered from five germplasm expeditions in which it was applied.

Materials and Methods

The CPCRI Protocol :

The protocol for coconut zygotic embryo culture developed at CPCRI has the following components: Direct field collection of 8 to 11 month old coconut embryos (Karun *et al.*,1993), short term storage (Karun and Sajini, 1994, Karun *et al.*, 1997), *in vitro* retrieval (Karun *et al.*, 1993, 1998) and *ex vitro* establishment (Karun *et al.*, 1999). It is characterized for short-term storage of embryos, rescue of immature embryos and higher rate of acclimatization of *in vitro* retrieved plantlets.

Expeditions in which CPCRI protocol applied

The CPCRI protocol was first applied during 1994 to collect 6 Pacific Ocean Island accessions maintained at World Coconut Germplasm Centre, Sipighat, Andaman Islands (Karun *et al., 2002*). Subsequently it was applied in five expeditions (Table 1). The embryos were collected from the sites using the portable inoculation hood. After surface sterilization, individual embryos were inoculated

into screw-cap vial (5 ml) containing 1.5- 2.0 ml of sterile water. The cultures were transported to CPCRI, Kasaragod in ambient condition. Number of days the cultures kept in sterile water is shown in Table 1.

The personnel deputed for germplasm collection were imparted with adequate training on field collection and inoculation of embryos. Number of days of training varied between 4 days to one week. Items required for the expeditions as per the checklist (Karun *et al., 2002*) were provided to the team, which included adequate number of sterilized vials containing sterile water.

Results and Discussion

Experts of plant breeding (to identify the desired accessions) and plant protection (to observe pest/disease incidence if any) usually constituted the germplasm expedition team. The team acquired sufficient skill for excising embryos and surface sterilization. A short duration training to meet this end seems to be inadequate. To improve the skills, long duration training is required in which the reasons for contamination could be identified and corrected. Alternatively, a skilled-person for embryo collection may be included in the team.

Loss of embryos before *in vitro* retrieval:

The viability of embryos collected in a germplasm expedition are lost mainly due to physical damage of embryos at the time of extraction and fungal and bacterial contamination during transit. Microbial contamination was more in physically damaged embryos. The percent damage of embryos noticed at the time of inoculation varied between 13 to 32%, except in expedition 4 and 5 (Table 2); the average loss was 25%. However, the contamination of embryos in expedition 4 and 5 was over 50% respectively due to mishandling of cultures during transit and improper surface sterilization. The severely contaminated embryos were discarded and those with mild bacterial contamination were individually washed in 70% alcohol and treated with 2 ppm tetracycline, thereafter surface sterilized with 0.01% mercuric chloride for 10 minutes, washed 2-3 times with sterile water, dipped in tetracycline solution and then inoculated into retrieval medium. Following this procedure, the loss of embryos collected in expedition 1 could be reduced from 23.24% to 7.97%. However, the seedlings derived out of the treated embryos were too weak to survive. Hence in subsequent expeditions, only mildly affected embryos were treated with tetracycline. Proper sealing of culture of vials after inoculation (individual vials must be sealed with para-film) and careful surface sterilization using correct concentration of sterilant could reduce the loss of embryos.

In vitro germination:

The percent germination of embryos varied between 54 to 82% (Table 2). This is similar to the results obtained in the laboratory studies. In an earlier report Blake (1993) was also obtained retrieval of 57% embryos collected from ten coconut accessions from the Solomon Islands and Indonesia.

Unlike in the laboratory studies, it was observed in the cultures of various expeditions that the growth of retrieved plantlets ceased during sub-culturing. Number of reasons could be attributed for this such as (i) mixing of embryos of different maturity stages as observed in expedition 1; (ii) repeated surface sterilization of embryos that were contaminated; (iii) use of common retrieval medium irrespective of the accessions.

No seedling could be retrieved out of 14 embryos of Pemba Yellow Dwarf from Mauritius, and only one plantlet survived out of 15 embryos of Coco gra accession collected from Seychelles having liquid endosperm. All the embryos collected from these two accessions were in immature stage. While germination of embryos of 8 month old onwards is possible, the growth of derived plantlet was poor compared to mature embryos (11

Table 1. Details of exotic coconut germplasm collected in the form of mature embryos since 1997

Expedition	Place of collection	Date of collection	No. of accessions collected	No. of embryos collected	*In vitro* active conservation (days)
I	Indian Ocean Islands				
	Mauritius	June 1997	6	427	45-50
	Madagascar		4	437	-do-
	Seychelles		5	502	-do-
II	Maldives	June 2000	8	921	20
III	Comoros	Aug. 2001	5	566	35-40
	Reunion	Aug. 2001	3	180	-do-
IV	Sri Lanka	Feb. 2001	4	746	9-18
V	Bangladesh	Dec. 2001	10	401	21-30

Table 2. Percent embryos retrieved (Embryos rescued on treating with tetracycline were excluded)

Expedition	Country	% Contaminated/damaged		Number Inoculated	Germination(%)	Plantlets in the (%) field
		At the time of receipt	Discarded			
I	Mauritius	23.19	12.41	374	73.86	5.35
	Madagascar	20.18	5.72	412	77.67	9.47
	Seychelles	26.35	7.37	465	66.82	6.67
II	Maldives	28.34	28.34	660	67.00	8.94
	Comoros	33.92	33.92	374	56.20	8.56
	Reunion	13.33	13.33	156	78.66	7.69
	Sri Lanka	53.08	53.08	350	54.00	35.71
	Bangaladesh	60.85	60.35	159	82.16	57.23

months old). The weak plantlets derived from immature embryos were not suitable for *ex vitro* establishment.

In Expedition- 1, large number of embryos were inoculated into a single vial (30-35 embryos/vial) and transported. In such cases the contamination was found to be more. For instance, all the 'bulk-inoculated' embryos of Dupays accession from Mauritius got were contaminated. Though the tetracycline treatment could rescue these embryos, only weak seedlings were obtained, probably because of repeated sterilization; no seedling could be established *ex vitro*.

The red and yellow dwarf accessions collected from Mauritius exhibited severe hyper-hydricity, cessation of growth after germination, senescence and necrosis *in vitro*). The collections from Mauritius included Guelle rose (having pink coloured husk) (Figs 1 and 2). Out of 50 embryos collected, 11 plantlets could be retrieved.

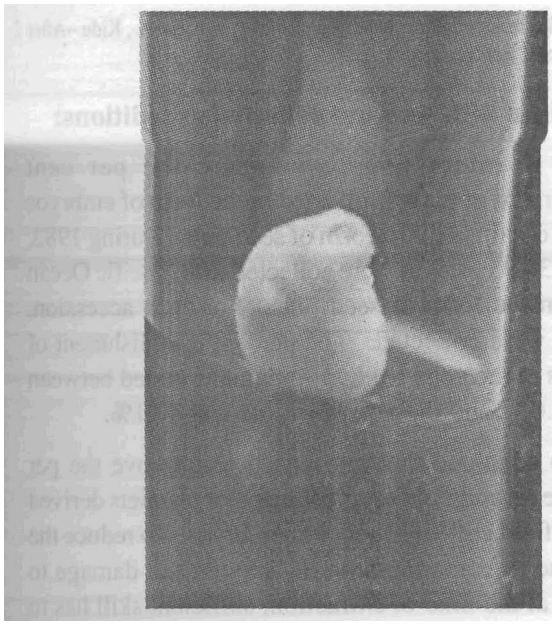

Fig.1. Guelle Rose accession collected from Mauritius- Germinating embryo with pink colour

Fig.2. Field established plantlet (Guelle Rose) at ICG-SA Kidu

In two expeditions (2 and 3), improper sterilization (either low concentration of sterilant or less time given for sterilization treatment) resulted in high percentage of contamination of embryos collected. The retrieval of plantlets in these two expeditions also suffered from the unforeseen adverse laboratory conditions that resulted in the loss of a large number of cultures in rhizogenesis medium. Again in Expedition - 5, more than 60% embryos showed bacterial contamination, due to improper surface sterilization. Thus, it is essential to have good training and practice in all the aspects of the protocol to achieve highest percentage of retrieval.

Ex vitro establishment and field planting:

The CPCRI protocol uses a potting mixture consisting of sterile soil, sand and coir dust in equal proportions. As a pre-cautionary measure before transplanting into the pots, the seedlings were treated with Bavistin (1 g/l), and thereafter with IBA solution (1000 ppm) for one hour each. High humidity was maintained initially by covering plantlets with polythene bags. Humidity was reduced gradually by providing perforations on the polythene bags and later lifting the bags during night and thereafter completely. Macronutrients of Eeuwens' Y3 (Eeuwens, 1976) medium were given to the plantlets once a month (Fig.3). The details of the *ex vitro* plantlets established in the field are shown in Table 2. Among the accessions collected in Expedition -1, the percent plantlets established in the field varied between 0 to 33%. The Tall accessions showed a higher rate of establishment (8.0%) compared to Dwarf accessions (2.10%). However, similar results were not observed in subsequent expeditions. For instance, in Expedition-3, the average field establishment of Tall accessions was 7.87% while it was 11.76% in Comoros Yellow Dwarf. Thus, the germination of embryos of different accessions was influenced mostly by factors prevailing at the time of collection than in the retrieval medium.

Fig. 3. Well established potted plantlets at shaded net house – Sri Lanka accession

Only few plantlets were lost at the time of hardening. The major problem was the dry rot disease, a total of 20 plantlets were lost due to this disease even after treating the infected plants with Dithane - M-45 (3 gm/l) once a week for 3 weeks. The initial symptom of the disease was yellowing of the tender leaves.

The percent plantlets established in the field from Expeditions 1 to 5 was 6.3, 6.4, 5.9, 16.8 and 22.7% of embryos collected and direct inoculated at the collecting site respectively. Figs. 4 and 5 show the field established plantlets at International Coconut Gene Bank for South Asia at Kidu farm of CPCRI in Karnataka.

Fig. 4. Field establishment of embryo derived plantlets at ICG-SA , Kidu – At the time of Field planting

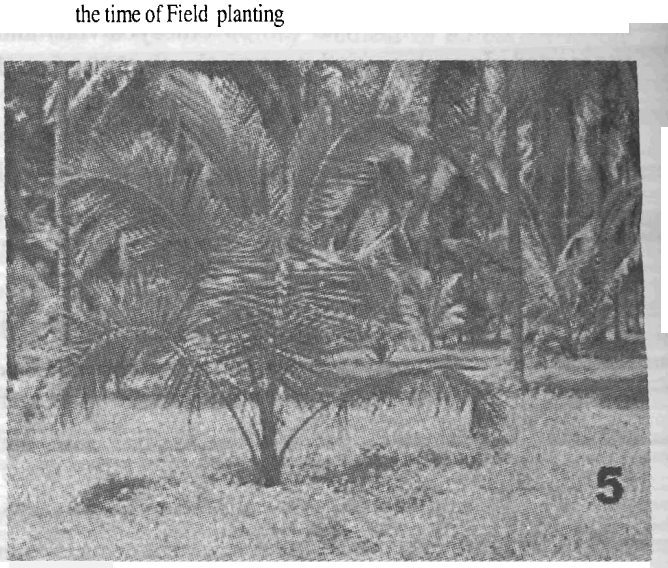

Fig. 5. Well established embryo derived plantlets at ICG-SA , Kidu –After 2 years of field planting

Comparison with seed nut collected expeditions:

It is interesting to compare the per cent establishment of plants collected in the form of embryos with that obtained in the form of seed nuts. During 1982, a total of 30 accessions were collected from Pacific Ocean Islands in the form of seed nuts. For each accessic 100 nuts were collected. The percent establishment seedlings in the gene bank at Andamans varied betwe 2.0 to 87.0 % and the average value was 47.1%.

To conclude there is a need to improve the per cent retrieval and field establishment of plantlets derived from the field collected coconut embryos. To reduce the losses due to contamination of cultures and damage to embryos at the time of extraction, sufficient skill has to be developed by persons involved in germplasm expeditions. Utmost care is to be taken while extracting

the endosperm plug containing the embyo from the nut, since the chances of damage is more there. By controlling the loss of excised embryos prior to inoculation into the retrieval medium, 30 to 50% plantlets per accession can be retrieved in the field.

Though embryos that got contaminated with bacteria could also be retrieved, destabilization of embryos poses problems in the later stages of development in the form of poor growth and abnormalities like browning and necrosis. Mixing of various maturity stages of nuts while harvesting should be avoided. As indicated in the laboratory studies, differential performance of accessions in the retrieval medium was noticed in all the five germplasm expeditions. However, no general recommendation for Tall and Dwarf types seems to be possible.

The observation on *in vitro* retrieval of embryos and *ex vitro* establishment suggest that about 300 to 400 embryos/accession need to be collected for successful field establishment of 100 plants in the gene bank. If collection of embryos is not a limitation the number may be 400 to 500 as suggested by Ashburner *et al.,* (1993).

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