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In vitro Coconut (*Cocos nucifera* L.) Embryo Culture in Bangladesh

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Abstract: Zygotic embryos of BARI Narikel 1, BARI Narikel 2, Srilankan Tall and Malaysian Dwarf were cultured on Y_3 basal nutrient medium. Germination percentages of embryos were recorded 93, 96, 89 and 40% in BARI Narikel 1, BARI Narikel 2, Srilankan Tall and Malaysian Dwarf respectively. Germination percentage was different due to the difference of physiological state of the embryos and/or difference in the genetic make up of the genotypes. Most of the embryos developed root and shoots simultaneously and survival rate was more then 60%. Significant difference in plant height, number of secondary roots, length of roots and transferable plantlet were found among the coconut genotypes.

Key words: Coconut embryo, *Cocos nucifera*, *In vitro* embryo culture

INTRODUCTION

Coconut (*Cocos nucifera* L.) is an important plantation crop in Bangladesh. It is commonly grown in homestead areas with mix species of plants for effective use of land[1]. It has a big domestic and international market due to its various uses. Every part of the palm and its fruits is used for human in Bangladesh. Coconut provides substantial environmental benefits. They help to reduce soil and beach erosion and act as windbreaks. So, the plantation of coconut plant in the new islands and seashores would be useful. The crown is used as a canopy and windbreak for shade loving intercrops like pineapple, coffee, banana and black pepper[2]. At present, there are about five million coconut palms in the country producing about 100 million nuts yearly from 30 thousand hectares of land[3]. Coconut cultivation needs to be developed in the country to alleviate human nutrition, increase farmers income, provide employment to rural women, save foreign exchange, protecting the coastal areas from the tidal waves, soil erosion and stabilize the country's watershed. For the better yield, high yielding varieties of coconut should be collect from the COGENT member countries.

In Bangladesh, a few indigenous (BARI Narikel 1 and 2) and exotic (Malaysian and Srilankan varieties, king coconuts and Nana) coconut promising varieties are available for cultivation. It is difficult to produce sapling true to type of those varieties due to high cross-pollination habit. Presently, Bangladesh have no reliable system of producing true to type plants. Therefore, the promising genotypes cannot be multiplied truly and efficiently. Moreover, collecting and exchanging of coconut germplasms is difficult and costly because of the considerable weight and size of the seed nut and rapid loss of viability. Excision of embryo provides a useful option to conventional methods by lowering transportation cost, overcoming storage problem and protecting most of the quarantine requirements[4]. Embryo culture offers a solution as it would allow the cheap transport of non-bulky embryos which could be grown into seedlings for transplanting on the field. *In vitro* culture of zygotic embryos of coconut has been practiced for a long time, with reports dating back to the early works of Cutter and Wilson[5], Abrahams and Thomas[6] and Ventura *et al.*[7]. Relative success has been obtained with this methodology for collecting, exchange and germplasm conservation and propagation of rare hybrids[4,8-13,23].

Different tall and dwarf varieties have been used for research and the development of their embryos into seedlings has been reported on various occasions[14-17,23,4]. This technology could help to promote the collection, exchange and conservation of promising germplasm[18]. Hence, the present study was undertaken to see the performance of *in vitro* coconut embryo culture in Bangladesh.

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MATERIALS AND METHODS

Initially, uniform mature embryos from varieties of BARI Narikel 1 and 2, Srilankan Tall and Malaysian Dwarf were collected for embryo culture. The embryo culture protocol developed by Eeuwens[20] known as Y_3 medium was followed. Rillo *et al.*[19] stated that coconut zygotic embryos grew and developed satisfactory in Y_3 nutrient formulation.

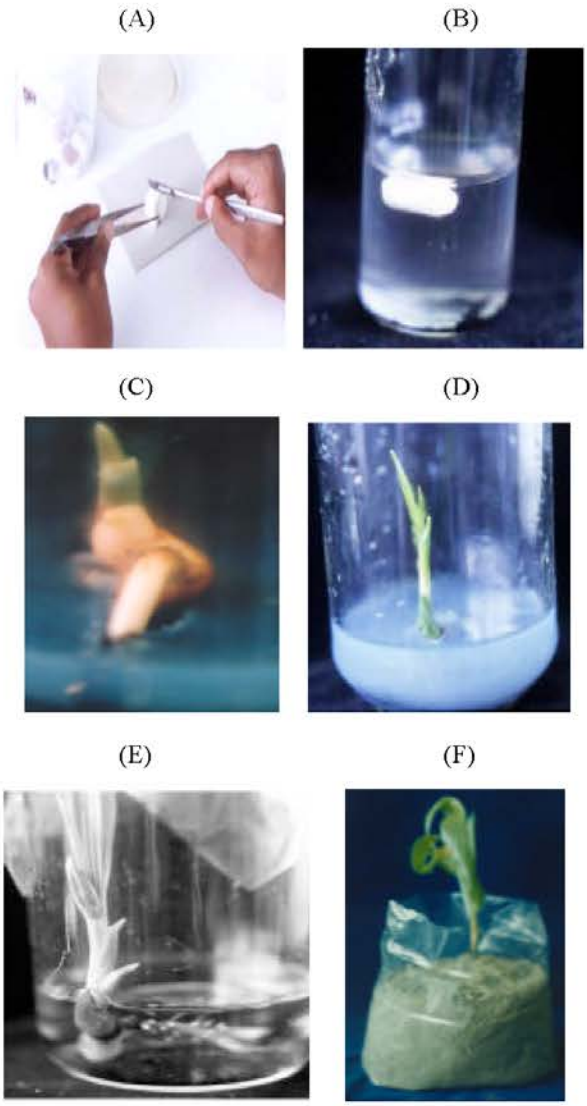

Fig 1(a-f): Different steps of coconut embryo culture
(A) Embryo separation
(B) Embryo in liquid medium
(C) Germinated embryo
(D) Growing of germinated embryo
(E) Rooted plantlet
(F) Plantlet

Embryo extraction: With the use of a cork borer (No. 10 or bigger) the embryos were extracted from the solid endosperm of the splitted nuts (Fig. 1a). The endosperm cylinders were pushed out from the cork borer using a piece of clean stick. These were collected in a clean container with plain water as medium.

Preparation of embryos for culture: The solid endosperm cylinders were washed with distilled water 4-5 times and quickly rinse was done by 95% ethanol. After washing 3-4 times with distilled water, endosperm cylinder was immerse in 100% commercial bleach (clorox) for 20 min. in a clean beaker. Rinse with sterile distilled water at least 5 times was done inside the laminar flow hood. The sterilized embryo was splitted carefully with sharp scalpel inside the laminar flow hood (Fig. 1b). The separated embryos were inoculated onto test tubes containing Y_3 liquid medium (Fig. 1c).

Culture conditions: Incubation cultures at 23-30^{0}C with approximately, 4000-5000 lux was done. Subculture to fresh medium at monthly interval and periodically checking for contamination was done.

RESULT AND DISCUSSION

Embryos of BARI Narikel 1, BARI Narikel 2, Srilankan Tall and Malaysian Dwarf were exhibited different germination rate. Results on percentage of germination, duration of embryo germination and *in vitro* growth and development of germinated embryos are presented in Table 1 and 2.

Most of the embryos showed better germination in variety BARI Narikel 1 (93.10%), BARI Narikel 2 (96.55%) and Srilankan Tall (89.33%) but the embryos from Malaysian Dwarf showed only 40.00% germination (Table 1). Taylor *et al.*[21] stated that Tall and Dwarf varieties showed different germination rates due to some factor or factors other than a varietal influence. Damasco[22] recorded 32.5- 45% germination in Malaysian Yellow Dwarf varieties. Within 4-6 weeks all of the embryos of BARI Narikel 1, BARI Narikel 2 and Srilankan Tall were germinated but the embryos from Malaysian dwarf has taken 16-18 weeks. Taylor *et al.*[21] obtained 48% germination after 12 weeks of culture in Malayan Red Dwarf Variety. Most of the embryos developed roots and shoots simultaneously but the growth rate was different. It may be due to the difference of physiological state of embryos during initial culture and or difference in the genetic make up of the variety. Some of the embryos of

Table 1: Performance of coconut embryo culture

	No. of embryo culture	Duration of germination (weeks)	No. of germinated embryo	% germination	% shoot formation only	% root formation only
BARI Narikel 1	58	04-06	54	93.10 (74.77)	9.25	3.7
BARI Narikel 2	87	04-06	84	96.55 (79.37)	7.14	0.0
Srilankan Tall	75	04-06	67	89.33 (71.00)	11.94	0.0
Malaysian Dwarf	10	16-18	04	40.0 (39.23)	0.0	0.0
Range	10-87	-	04.0-84.0	40.0-96.55	0.0-11.94	0.0-3.7
$\bar{x}$	57.50	-	52.25	66.09	7.08	0.98
SD	29.30	-	29.81	18.23	4.42	1.60

Data within parenthesis represent the angular transformed value.

Table 2: *In vitro* growth of coconut embryos and seedlings

	Plant height (cm) at 6 month	No. of leaves/ in vitro plant	No.of roots/*in vitro* plant		Length of roots		Transferable plantlet
			Primary	Secondary	Primary	Secondary	
BARI Narikel 1	14.45±1.59	3.5±0.5	1.0±0.0	9.25±3.27	9.06±0.72	3.58±0.42	34.0
BARI Narikel 2	14.76±1.50	3.0±0.0	1.25±0.43	8.33±1.58	4.25±0.71	3.33±0.35	52.0
Srilankan Tall	17.00±2.49	3.25±0.43	1.50±0.873	22.75±7.29	4.22±1.19	3.60±0.9	48.0
Malaysian Dwarf	4.60±0.38	3.0±0.0	1.0±0.0	0.0±0.0	4-3±0.37	0.0±0.0	4.0
Range	4.6-17.0	3.0-3.50	1.0-1.5	0.0-22.75	4.22- 9.06	0.0-3.6	4.0-52.0
$\bar{x}$	12.70	3.19	1.19	10.08	5.46	2.63	34.5

BARI Narikel 1, BARI Narikel 2 and Srilankan Tall produced 9.25, 7.14 and 11.94% shoot only. These embryos did not produced any roots. On the other hand, 3.7% embryos of BARI Narikel 1 produced roots only with very poor shoot development. Survival rate was more than 60%. Contamination was high at 1st and 2nd subculture compare to initial culture. At the end of 6.0 month plant height varied from 4.60 to 17.0 cm where the highest was in Srilankan Tall (17.0 cm) and lowest was in Malaysian Dwarf (4.6 cm). No. of leaves and no. of primary roots were more or less similar in BARI Narikel 1, BARI Narikel 2 and Srilankan Tall (Table 2) but in Malaysian Dwarf no. of leaf and no. of root was very less due to late germination. Highest no. of secondary roots were recorded in Srilankan Tall (22.75) followed by BARI Narikel 1 (9.25) and BARI Narikel 2 (8.33). Difference among the length of primary roots were found in BARI Narikel 1, BARI Narikel 2, Srilankan Tall and Malaysian Dwarf. Highest length was recorded in BARI Narikel 1 (9.06 cm) followed by, Malaysain Dwarf (4.3 cm) and BARI Narikel 2 (4.25 cm). Lowest length was recorded in Srilankan Tall (4.22 cm). Secondary root length of BARI Narikel 1, BARI Narikel 2 and Srilankan Tall were very close to each variety (Table 2). Transferable plantlets were highest in BARI Narikel 2 (52.0) followed by Sri Lankan Tall (48.0). Lowest was in Malaysian Dwarf (4.0). Germination and growth pattern of cultured embryos are shown in Fig. 1.

From the above result, *in vitro* coconut zygotic embryo culture on Y_3 medium is suitable for plantlets production. For the better growth and development of shoots and roots some of shooting and rooting hormone may be incorporated with the medium.

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