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A COST EFFECTIVE MEDIUM FOR PRODUCING EMBRYO CULTURED COCONUT PLANTS

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ABSTRACT

Zygotic embryo culture of coconut is a useful research tool employed in various fields of crop science. In the present study, the feasibility of reducing the production cost of embryo-cultured coconut plants was explored. In separate experiments, Analar grade potassium chloride, sucrose and double distilled water in the Y_3 medium were substituted with Muriate of Potash (fertilizer grade potassium chloride), table sugar and tap water, respectively. Furthermore, embryos were cultured in a growth medium devoid of hormones (BAP and 2,4-D).

The results indicated that low cost ingredients can be used in culture media without affecting the germination and subsequent growth of coconut embryos. The results also revealed that incorporation of hormones to the culture medium is not essential. The findings will contribute to reducing the cost of producing coconut plants *in vitro*.

INTRODUCTION

Zygotic embryo culture of coconut (*Cocos nucifera* L.) is a useful tool for research in various fields, such as germplasm collection, exchange and storage (Assy-Bah *et al.*, 1987; Karunaratne, 1988), embryo rescue (De Guzman, 1970; Balaga and De Guzman, 1971; Karunaratne, 1987), disease transmission (Orense and Pacumbabba, 1988), screening for disease resistance (Rillo *et al.*, 1988) and drought tolerance (Karunaratne *et al.*, 1991).The first published research on coconut zygotic embryo culture was carried out by Cutter and Wilson (1954). Since then much work has been carried out by a number of scientists to improve *in vitro* culture of coconut zygotic embryos and the technique has been perfected. According to our knowledge, in most of the investigations on zygotic embryo culture of coconut, culture media contained analytical grade chemicals, which are costly. Low cost ingredients such as fertilizer grade chemicals and table sugar have been used in several tissue culture systems to reduce the production cost (Ganapathi *et al.*, 1995; Purnima, 1997; Sujatha and Chandran, 1997). In coconut embryo culture, the possible use of tap water instead of glass distilled water (Areza *et al.*, 1995) and refined table sugar instead of Analar grade sucrose (E Rillo personal communication) in the culture medium, was reported recently. These findings encouraged further

work on cost reduction in producing embryo cultured coconut plants. The aim of the present study was to determine the feasibility of reducing the production cost of embryo cultured coconut plants by using low cost ingredients and eliminating hormones in the growth medium.

MATERIALS AND METHODS

Mature zygotic embryos were collected from freshly harvested nuts (12-13 months postanthesis) of Sri Lanka Tall coconut. The embryos were sterilized, cultured and incubated as described by Karunaratne *et al.* (1985). In separate experiments, Analar grade potassium chloride (KCl), sucrose and double distilled water in Y_3 medium (Eeuwens, 1978) were substituted with Muriate of Potash (fertilizer grade KCl), table sugar and tap water respectively. All the other chemicals used for media preparation were of Analar grade. The possibility of eliminating hormones (BAP and 2,4-D) in the growth medium was also tested by growing embryos in the growth medium devoid of hormones. The standard growth medium containing Analar grade chemicals and hormones was used as the control.

During the course of germination and development of the cultured embryos, the numbers of germinated embryos, developing into plants, photosynthetic leaves per plant, primary roots per plant and plant height were recorded.

Eight replicates each consisting of 20-25 embryos were used for each treatment. A completely randomized design was used in all the experiments. The data was analyzed using the analysis of variance. The percentage data was analyzed using inverse sine transformation.

RESULTS AND DISCUSSION

The results indicated that quality of KCl, sucrose and water (Tables 1 & 2) did not affect the germination and subsequent growth of coconut embryos. It is note worthy that although commercial grade chemicals were used in similar quantities as analytical grade, it did not have any negative effect on embryo germination and plant growth indicating that any impurities present in commercial grade material had no deleterious effect. Reduction of production cost of many crops including *Melia azedarach* L. (Sujatha and Chandran, 1997), *Brassica campestris* (Okuno et al., 1996), *Musa* spp. (Ganapathi et al., 1995) and *Zingiber officinale* (Sharma and Singh, 1995) has been achieved by substituting Analar grade sucrose with table sugar. Furthermore, Malemnganha *et al.* (1994) have tested a medium prepared by using a commercial fertilizer formulation, table sugar and coconut water for *Dendrobium* spp. embryo culture. The successful use of tap water instead of

distilled water in micropropagation of *Musa* spp. (Ganapathi *et al.*, 1995), *Solanum tuberosum* (Purnima, 1997) and *Cocos nucifera* (Areza *et al.*, 1995) has also been reported. Thus the above studies have shown that commercial grade chemicals could be used successfully in *in vitro* culture and the production cost of *in vitro*-raised plants could be reduced significantly. The results of the present study are in agreement with these findings.

The use of non-ionic water instead of double distilled water and refined table sugar instead of Analar grade sucrose has resulted in slight decrease in secondary metabolites content of cell cultures (Zheng *et al.*, 1982; Jianping *et al.*, 1996). However, the decrease was not significant when compared to the cost reduction achieved by using cheaper chemicals.

The results of the present study revealed that coconut embryos could be germinated and grown successfully in media devoid of hormones. Furthermore, the number of leaves per plant developed in hormone-free medium was significantly higher than that of plants developed in the control medium (Table 3). This would be beneficial as plants having more leaves will have a better chance of survival during acclimatization. The control contained 0.1 µM 2,4-D, 5 µM BAP and 0.25 % activated charcoal. Activated charcoal is added to the culture medium to reduce necrosis as it adsorbs toxic components released from tissues. However the presence of activated charcoal in a culture medium results in the reduction of freely-available hormones due to the adsorption of hormones by activated charcoal (Ebert and Taylor, 1990). As the adsorption capacities of different types of charcoal differ, the level of freely-available hormones present in the medium depends on the type of the activated charcoal. However, Banaobra III *et al.* (1998) reported that any brand of activated charcoal could be used satisfactorily for *in vitro* culture of coconut embryos. This indicates that the level of hormones is not critical in coconut embryo culture. Considering the above facts, and the results of the present study, it can be concluded that incorporation of the hormones (2,4-D and BAP) to the culture medium is not essential for growth of coconut embryos and a hormone-free medium can be recommended for coconut embryo culture.

The results of the present study indicated the feasibility of using low cost ingredients in culture media and using media devoid of hormones for coconut embryo culture. Thus, the findings will contribute to reduction of cost in producing coconut plants through embryo culture.

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Table 1: Effect of low cost chemicals on growth of *in vitro*-cultured coconut zygotic embryos

Treatment	Germination (%)	Plant development (%)	Plant height (cm)	Number of leaves per plant	Number of primary roots per plant
Control	78.9	54.1	9.6	1.4	2.4
Sc	83.9	53.0	8.9	1.4	2.3
Kf	79.8	45.0	9.7	1.4	2.2
Sc + Kf	79.8	62.8	9.4	1.4	2.1
LSD (p=0.05)	NS	NS	NS	NS	NS
CV (%)	16.2	20.9	21.7	19.2	21.1

Growth measurements of plants were recorded after four and a half months of culture. Control = Growth medium with Analar grade chemicals, Sc = Growth medium with table sugar, Kf = Growth medium with Muriate of Potash (fertilizer grade KCl), Sc+Kf = Growth medium with table sugar and Muriate of Potash (fertilizer grade KCl) NS = Not significant.

Table 2 : Effect of water quality on growth of *in vitro*-cultured coconut zygotic embryos

Treatment	Germination (%)	Plant development (%)	Plant height (cm)	Number of leaves per plant	Number of primary roots per plant
Control (deionized water)	73.4	75.8	24.8	1.8	2.0
Tap water	73.8	70.4	23.9	1.9	1.8
LSD (P=0.05)	NS	NS	NS	NS	NS
CV (%)	18.5	28.6	7.7	8.4	9.9

Growth measurements of plants were taken after 6 months of culture.
NS = Not significant.

Table 3 : Effect of hormones on growth of *in vitro*-cultured coconut zygotic embryos

Treatment	Germination (%)	Plant development (%)	Plant height (cm)	Number of leaves per plant	Number of primary roots per plant
Control (with 0.1 µM 2,4-D and 5 µM BAP)	68.2	78.5	26.2	1.9	2.0
Medium without hormones	81.9	76.6	29.4	2.2	2.0
LSD(P=0.05)	NS	NS	NS	0.2	NS
CV (%)	21.9	8.8	10.4	8.7	11.8

Growth measurements of plants were taken after six months of culture.
NS = Not significant, S = Significant.