SHORT TERM in vitro PRESERVATION OF COCONUT SEED MATERIAL: A METHOD TO FACILITATE FIELD COLLECTION AND TRANSPORT OF COCONUT GERMPLASM

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SUMMARY

High cost of transport and the short storage fife of recalcitrant seednuts are two major obstacles to the effective field collection and exchange of coconut germplasm. An *in vitro* method involving the culture of zygotic embryos of coconut was developed to overcome these problems. The excised embryos were initially subjected to a slow growth phase by culturing in an agar based medium containing nutrients barely sufficient for their survival. After 2, 3 and 5 months in this medium, the embryos were transferred to the gerinination or the development medium for resumption of growth and plant development. 53% of the zygotic embryos preserved in this manner for a period of 2 months produced normal plants when transferred to the gennination medium. The percentage germination dropped to 32 after 5 months storage in the survival medium. The prolonged low temperature storage of cultures (10^0 and 4^0C for three months), caused irreversible damage to the coconut embryos and no plants were formed when transferred to the germination medium.

The advantages of the technique are that a large number of 'seednuts' can be transported free of pests in a small container and requires no change in the culture medium during the preservation period. Unlike the germination medium, the survival medium is agar based and hence the risk of containination is low. The non gerridnators can be identified and rejected prior to the germination phase.

INTRODUCTION

Coconut is one of the most useful and important plantation crops in Asia and the Pacific region. It ranks among the top five oil producing p1mt species in the world. Coconut is monotypic but a range of varieties and forms could be seen distributed over the coconut growing area of the world (Yan Fremond et at (l966). For production of improved planting materials, superior parental lines are selected based on vegatative and yield characters of these varieties and fonns and hybridized with similar individuals by controlled pollination. Local germplasm is used extensively for this purpose by many countries. International (and sometimes local) movernent of coconut germplasm and planting materials for crop improvement programmes is either prohibited or restricted due to numerous pests and diseases of considerable importance that are not yet established in some countries of the region or have only restricted distribution within a country (Nayar 1982).

Another major factor limiting the movernent of the coconut seed is its tendency to loose viability during the course of collection and transport. Coconut seed is recalcitrant and has a short storage life of about two months. Thus, an efficient method to maintain seed viability has to be developed to facilitate field collection and long distance transport. Air lifting large quantities of bulky seednuts and planting material is also not economical. These problems can be alleviated to a considerable extent by utilizing the embryo culture technology for transport of coconut seed and planting material. But, the coconut embryo appears to grow better in the liquid medium than in a base solidified with agar. The technique thus needs to be modified so as to eliminate possible aging and drying up of cultures, spilling, contarnination etc during long expeditions of germplasm collection.

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An investigation was undertaken by the Coconut Research Institute with the objective of developing a low cost but effective in vitro (short term) preservation or storage method to facilitate collection and long distance transport of germplasm and planting materials of coconut. The preliminary findings of this investigation have been reported earlier (Karunaratne *et al* 1985). The present paper is a further account of this investigation.

The use of the liquid development medium (Karunaratne *et al* 1985) for collection of coconut germplasm was reported recently by Sossou *et al* (1987). This involves no preservation of coconut embryos as such but explanting of zygotic embryos in the field and direct culture in the liquid medium, placed in a specially designed container called the Sossou flask.

MATERIALS & METHODS

The embryos of the three colour forms of *Cocos nucifera L, var. nana,* namely *pumila*, *regia* and *eburnea* were obtained from mature nuts, immediately after harvesting and disinfected using the technique described earlier (Karunaratne *et al* 1985). The embryos, after disinfection were transferred to screw capped culture vials (2 per vial with cotyledon and half buried) containing 10 ml of the survival medium and incubated in the dark at 28^{O}, 10^{O} and 4^{O}C. The survival medium was agar based (0.8%) and consisted of one-half strength of macro and micro salts and vitamins and growth factors of the development medium (a modification of Eeuwen's, 1978), 60 g/l sucrose and 0.25% activated qharcoal. After 2, 3 and 5 months preservation in the survival medium, the embryos were transferred to the development medium (Karunaratne *et al* 1985) for germination. The control treatment consisted of culturing fresh embryos directly in the development medium. About 100 embryos were used for each treatment.

RESULTS AND DISCUSSION:

The embryos preserved in the survival medium at temperatures 28' 10' and 4'C showed no signs of visible growth during the extended periods of storage (Fig. 1). When transferred to the liquid germination medium the viable embryos sprouted immediately. The culture medium was changed every third week during the course of development of the seedlings. The seedlings produced 3 - 4 photosynthetic leaves in 5 months (Fig. 2).

The results presented in table l show the average percent gennination achieved from the embryos, after 2, 3 and 5 months of preservation in the survival medium at 28^{O}C. No significant differences in the germination rates were observed among the three colour fonns of the var nana.

Table 1. Percent germination of coconut embryos of var nana after extended periods of storage in the survival medium at 28^{O}C.

Storage period in the survival Medium (months)	Percent germination (average) in the development medium		
	Eburnea	Pumila	regia
0 (control)	63.08	60.80	61.35
2	54.35	53.60	50.2
3	40.72	40.0	41.27
5	32.30	32.0	30.35

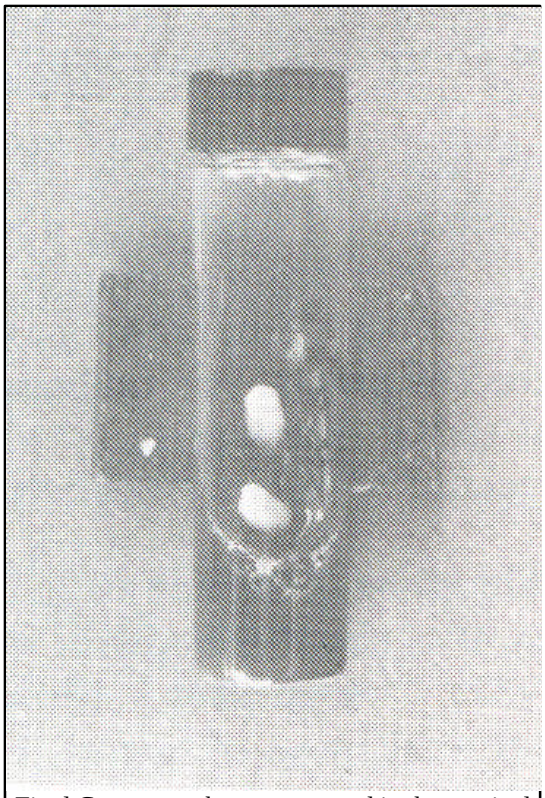

Fig. l Coconut embryos preserved in the survival medium

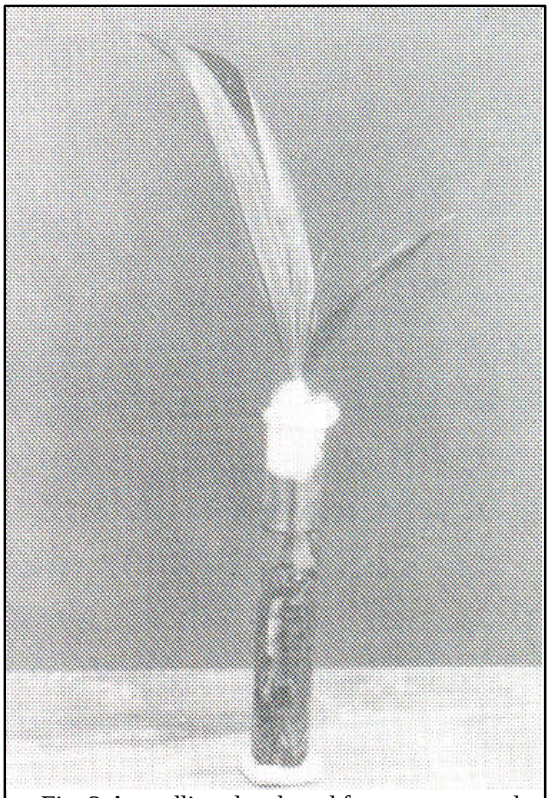

Fig. 2 A seedling developed from a preserved embryo

The percentage germination of the preserved embryos dropped gradually during extended periods of storage. About 53% of the embryos preserved for a period of 2 months resumed growth when transferred to the survival medium. This figure dropped to about 40% after three months and to 32% after 5 months of preservation. The percentage germination in the control which consisted of culturing fresh embryos directly in the development medium, was 62.

By careful examination of the preserved cultures, it was possible to identify the non germinators and the embryos damaged while in culture and consequently lost their capacity to germinate. The non germinators, retained their fresh appearance throughout the preservation period. However, the minute root end of the embryo pr6per was observed to be damaged pennanently and tumed brown (Fig. 3). This presumably was the main reason for non-germination. It was possible to identify such embryos (non genninators) easily and discard prior to the second culture (germination phase) thus saving on labour and the culture medium.

In an attempt to extend the preservation period and to improve the regeneration rate subsequently, the embryos cultured in the survival medium were stored under two low temperature conditions, 10^{O} and 4^{O}C for a period of 3 months. These embryos on transfer to the development mediurr produced abnonual structures from the cotyledon (Fig. 4) and normal seedhngs were never fonned. The root end of the embryos tumed brown as in the case of non genninatcrs in the previous experiment. This indicates that prolonged low temperature storage has a detrimental effect on the growth of embryos of this highly tropicalised palm. This also casts some doubt on the viability of seednuts that may be stored under low temperature conditions, during long distance transport.

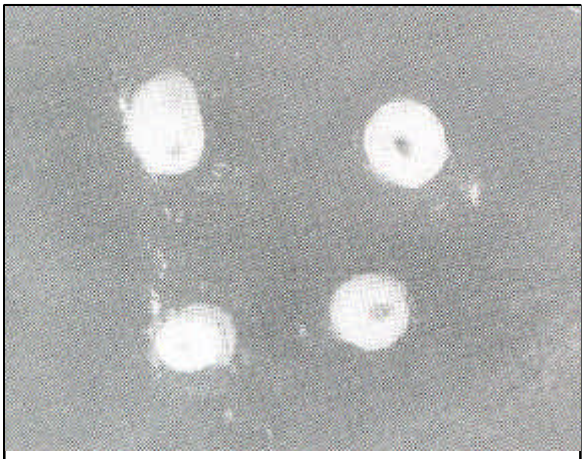

Figure 3. Non germinators Note the damaged root end (black spot)

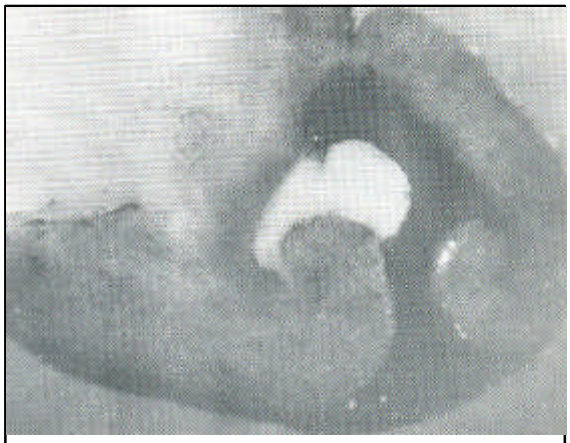

Figure 4. Abnonnal structures developing from an embryo preserved at 4^0C for 3 months and cultured in the development medium.

The technique of preserving embryos by slow growth has a number of advantages. Firstly, no specific growth retardants are used in the survival medium. The growth of the embryos is suppressed by reducing the level of nutrients in the original development medium to a bare minimum. The reduced or slow growth may also be attributed to the limited availability of water in the agar based medium containing high sucrose (60 g/1). Thus, the question of possible adverse effects of growth retarding substances on embryo growth does not arise. Secondly, the survival medium is agar based and a large number of embryos can be transported easily and very economically. About 10-20 coconut 'seednuts' can be easily stored in a 10 cm diameter petridish containing 10-15 ml of the survival medium(Fig. 5). There is no necessity for changing the culture medium during the storage period. The non germinators can be identified and discarded prior to the germination phase. Lastly, the germination rates of 53, 40 and 32% after 2, 3 and 5 months of preservation respectively are acceptable compared to the heavy losses that may occur through perishing, high cost of transport, labour involved in handling and the possible introduction of pests when seednuts/seedlings are transported.

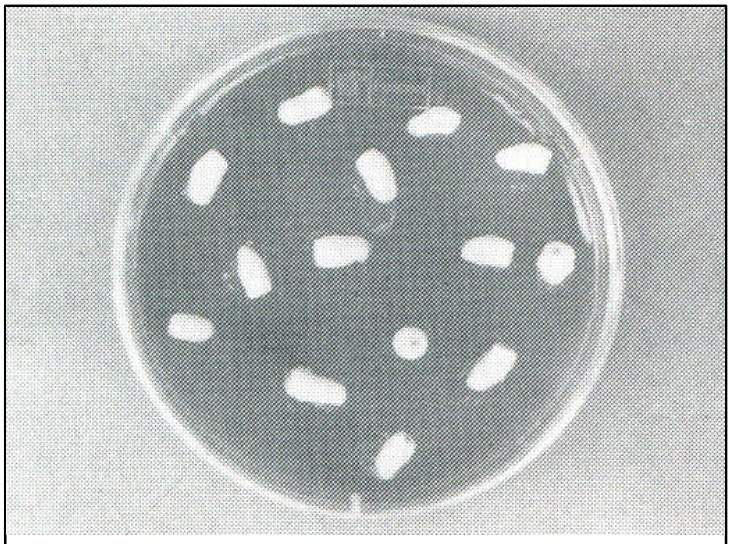

Figure 5. Coconut "seed" material in a 10 cm diameter petridish containing survival medium.

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