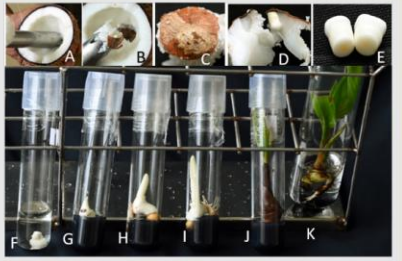

A. positioning the cork borer for embryo excision (the embryo is seen below the soft eye)
B. Scooping out endosperm plug
C. The endosperm plug with the projection at the top beneath which embryo is placed
D. Endosperm plug cut into two revealing the embryo
E. Embryo
F. Initial inoculation in liquid media
G,H. Different growth stages of the embryo
I. Development of proper root and shoot in the embro
J. Embryo cultured plantlet ready to be transferred to liquid media
K. Plantlet with proper shoots and roots ready for potting
L. *In vitro* hardening

M: Plantlet in net house

COCONUT ZYGOTIC EMBRYO CULTURE

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Introduction

Coconut (*Cocos nucifera L.*) is a recalcitrant crop. The conventional method of coconut propagation is through the nuts. The nuts are heavy and bulky in nature and lack dormancy. These factors act as constrains for the transport of the propagation material for germplasm exchange. Hence embryo culture was used as a strategy for international germplasm exchange together with an added advantage of averting the spread of diseases and pests. ICAR-CPCRI has developed an embryo culture protocol for the extraction as well as the *in vitro* development of the embryos.

Embryo excision:

The coconut embryo is found under the soft eye of the nut. The embryo is extracted with the help of a cork borer which is usually a steel pipe with serrated ends and attached with a handle. The borer is inserted into the endosperm covering the embryo and with a twisting motion the endosperm plug together with the embryo is extracted. This plug is surface sterilized with mercuric chloride @ 0.01% for 3 minutes and washed in running water. The embryo is obtained by splitting these plugs into half.

The extracted embryos are again sterilized with 20% sodium hypochloride for 15 minutes andthoroughly washed insterile water. These embryos are inoculated individually to sterile culture medium.

Media and culture conditions:

Initial inoculation

Eeuwens Y3 medium + sucrose (6%) +NAA (0.5mg/l) +BAP (0.5mg/l) + charcoal (2g/l) (dwarf accessions)
Eeuwens Y3 medium + sucrose (3%) +NAA (0.5mg/l) +BAP (0.5mg/l) + charcoal (1g/l) (tall accessions)
The embryos are inoculated in solid retrieval medium (Initial inoculation media + 7% Agar) and incubated in the dark. Subculture is done at monthly intervals until the embryos germinate. Average germination time for mature embryos is 20-25days for dwarfs and 35-40 days for talls.

Plantlet regeneration:

Four months after inoculation, the germinated embryos with two leaves and primary root is transferred to liquid rooting medium (Eeuwens Y3 medium + sucrose (3%) +NAA (1mg/l) +BAP (5mg/l) + charcoal (2g/l)) .

Subculture is done at monthly intervals. As the size of the plantlet increase, the bigger, wide mouth tubes are to be used.

Acclimatization

Plantlets are covered with polythene bags and kept indoor at room temperature with artificial light for 2-3 weeks. After two weeks, plantlets are hardened by gradually perforating the polyethene bags to reduce humidity. After two weeks, the polythene bags are removed. Water spray can be given to keep the potting mixture moist.
The plantlets are transferred to bigger pots and kept in a net house with 50% shade. After 3-4 months, the plantlets are transferred to a big polythene bag containing soil and organic manure.
The total duration of transfer from pot to polybag is 5-6 months. Irrigation and application of a recommended dose of fertilizer are carried out. After 4-5 months, plantlets can be transferred to the field.