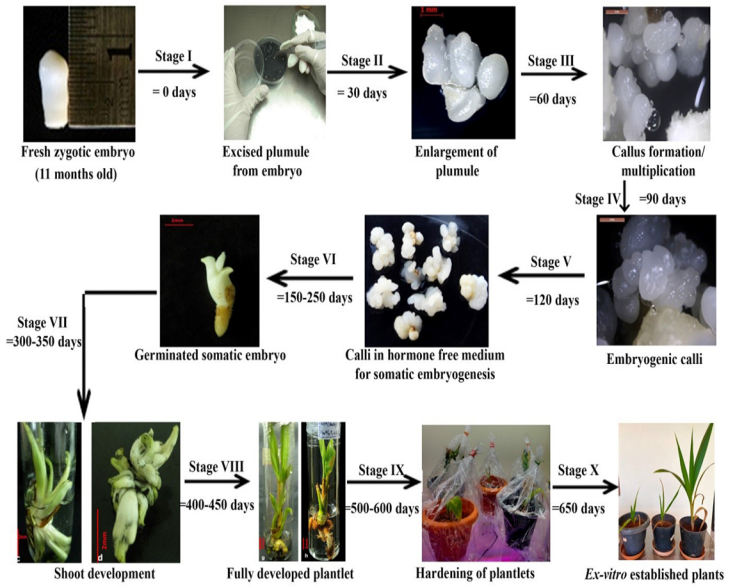
Flowchart of somatic embryogenesis via plumular explant of coconut is illustrated in figure 2.



Merits of Clonal propagation using plumular explants:

The development of an efficient method of cloning coconut using plumular explants offers a potential for the development of a long-term *in vitro* means of conserving significant coconut germplasm by cryopreservation of plumular explants. Mass multiplication of elite palms selected on the basis of resistance to root (wilt) diseases of coconut is possible using plumular explants. Plumule cultures can be used for rapid multiplication of proven coconut hybrids.

PLANTLET REGENERATION VIA SOMATIC EMBRYOGENESIS FROM COCONUT PLUMULAR EXPLANTS

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Introduction

In tissue culture, selection of explants is considered as a key element for a successful outcome.

The embryonic tissue or plumule of coconut embryo is a responsive explant for coconut tissue culture. Plumules are extracted from the coconut zygotic embryos (fig. 1) and inoculated into callus initiation medium.

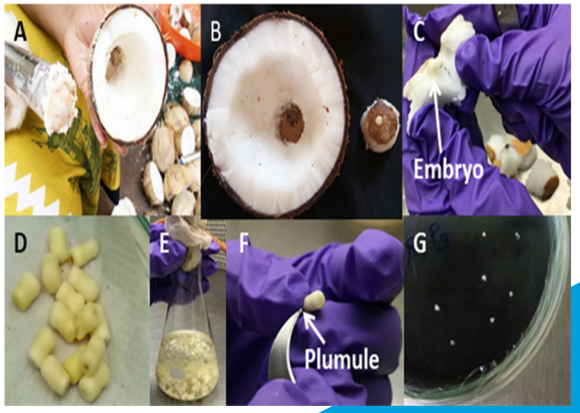

Figure 1: Process of excision of plumule and inoculation

A: Excising endosperm plug using scooper
B. Excised endosperm plug
C: Extracting endosperm from the endosperm plug
D: Extracted embryo
E: Embryo sterilization (20% sodium hypochlorite for 15 minutes)
F: Plumular extraction using sickle shaped blade
G: Plumule inoculated into media

Culture medium:

Callus initiation medium

Basal medium: M72 or Y3
Auxins: 2,4-D (16.5g/l) or Picloram (24.1mg/l)
Carbohydrate source: Sucrose (3g/l)
Gelling agent: Agar (7g/l)
Other essential components: Activated charcoal (1g/l)
pH: 5.8

Subculture schedule:

Subculture is carried out at monthly intervals to serially reduced concentration of auxin (2,4-D (mg/l) : 16.5 ➡ 10 ➡ 5 ➡ 1➡ Hormone free; Picloram (mg/l) : 24.1 ➡ 12.05 ➡ 6.025 1 ➡ Hormone free) .

The polyamine spermine (20mg/l) is added from the second to fourth subculture. The cultures were incubated in dark condition at temperature of 27±2°C. Once the somatic embryos are formed the cultures are transferred under warm-white fluorescent light with a 16 hour photoperiod. Somatic embryos are transferred initially to hormone free Y3 media and then to medium with BAP 4mg/l. Rooting was induced either by incubation in Y3 media supplemented with IBA (4mg/l) alone or in combination with NAA (1mg/l) for a period of 3-4 weeks.

Acclimatization:

Plantlets with minimum 2-3 opened leaves and 3-4 primary roots with well developed secondary roots were successful for cent percent ex vitro recovery in pots. Such plantlets were removed from the culture tubes and washed with sterile water. Before transferring to pots, the plantlets were treated with carbendazim (1%) and thereafter with IBA solution (1g/L) for an hour. The potting mixture consisted of sterilized soil, sand and coir dust in equal proportions. Initially, the plantlets were covered with polythene bag for two weeks. Gradually the bags were perforated to reduce humidity and later the bags were removed during the night. After 4 weeks, the bags were removed completely. Well developed plantlets were acclimatized under green house conditions and then successfully established in the field .