Rooting stage

Vigorous shoots of length 5-10cm with 3-4 leaves are transferred to ½ Y3media supplemented with Indole Acetic Acid (1mg/L), Naphthalene Acetic Acid (2mg/L) and Indole Butyric Acid (1mg/L) sucrose (40g/L), activated charcoal (1g/L). Cultures are dispensed to test tubes containing rooting media capped with cotton plugs. Bottom portion of test tubes are to be covered with Aluminium foil for avoiding degradation of auxins. Sub culturing is done 6 weeks interval to the same media composition except sucrose for which the concentration is gradually reduced.

Acclimatization

Sterile hardening

Wash the plantlets with roots and shoots in tap water to remove traces of media. Place the plantlets in a beaker containing 50 ml Carbendazim solution (1g/L) for 1 hourfollowed by in IBA solution (1000pm) for 30 minutes to 1 hour depending upon root formation. Prepare potting mixture containing sterilized cocopeat and perlite in 3:1 ratio. Whilemixing add one spatula humus mix and fill in a disposable plastic cup (with few holes atbottom)or small pot.Place plantlets in a storage container and seal the container using klin in wrapping film for developing high humidity. Provide nutrition (Y3 macro) and irrigation once in 2-3 weeks and incubated at 27 ± 2°C and light intensity of 2500 lux for 16 hours.

After 30 -45 days, transfer the sterile hardened plant to a bigger pot containing fresh potting mixture (cocopeat and perlite in 3:1) and cover it with polythene bag for 2-4 weeks and keep it indoor. Provide nutrition (Y3 macro) and irrigation once in two weeks. Harden the plants by making small perforations on the polythene bags and gradually remove the polythene bag.

Hardening

Pot the hardened plant in polybag containing sand : soil : cocopeat in 1:1:1 and keep in green house with 50% shade.

Fig. 3. Hardening

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Immature inflorescence culture of coconut

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Introduction

Immature inflorescences of coconut are considered as one of the promising explants for direct *in vitro* regeneration of coconut. The advantage of using immature inflorescence is that it allows multiplication of adult palms having desired characters like high yield and disease resistance for producing true-to-type plants

MATERIALS

- Immature inflorescences of coconut of outer spathe length 2cm to 12 cm (corresponding to the developmental stages -6 to -11, with zero corresponding to the mature unopened inflorescence)
- Culture media Y3 and ½ MS
- Ethanol 70%
- Laminar air flow cabinet, conical flasks, beakers, scalpel, surgical blades, cotton etc.

MEDIA PREPARATION

- For preparation of basic culture media Y3 (Eeuwens, 1976) and ½ MS (Murashige and Skoog, 1962
- Each of different media combination, supplement the basic media with growth regulators and other components
- Once all components are mixed adjust the pH to 5.75 before autoclaving at 15 lbs at 121° C for 21 minutes

METHODOLOGY

Collection of Explant

Select mature palms of more than 25 years old (having high yield, disease resistance or any other desired attributes). Cut the palm down and detach the leaves, bunches, petioles etc to expose the immature inflorescence including those have already opened and also unopened

- Select the unopened immature inflorescence of developmental stages (-6 to -11). Care should be taken to collect the inflorescences without any damage
- Rinse the inflorescences with distilled water and place the inflorescences in a plastic bag and immediately bring to lab

Fig.1. Immature inflorescence collection and explant preparation

Culture initiation

The cultures are then incubated under complete darkness at 27 ± 2°C and sub cultured to same medium every 4-6 weeks depending upon the growth of cultures for 32 weeks

Fig.2. Growth of Rachillae bits in dark

Multiplication of vegetative buds

The clusters of vegetative buds are cultured on to 250 ml conical flasks containing 100ml ½ MS media supplemented with Naphthalene Acetic Acid (1mg/L), 6- Benzylaminopurine (1mg/L), glutamine (100mg/L), sucrose (40g/L), activated charcoal (1g/L) and solidified with agar (7g/L). The cultures are initially incubated under diffused light (100 lux) for 4 weeks. The cultures gradually turn green and green cultures are kept at a light intensity of 2500lux for a period of 16 hours.

Elongation stage

The multiple shoots which could be easily detached from clump from the previous treatments are transferred to Y3 media supplemented with 2ip (5mg/L) and Naphthalene Acetic Acid (1mg/L) sucrose (40g/L), activated charcoal (1g/L) and solidified with agar (7g/L). Sub culturing is done to the same media at 4 weeks interval. The cultures are maintained at 27 ± 2°C and light intensity of 2500 lux for 16 hours.