Embryo Culture Protocol for Collection and Exchange of Coconut Germplasm

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Coconut, being a recalcitrant species with regard to the storage of seeds, problems are often encountered in transportation of seed nuts from far off places. Large size of the seed, water content and stringent phytosanitary requirements are the major problems in collection, transportation and storage of coconut germplasm. The success of *in vitro* germination of coconut zygotic embryos provides an alternative way of transportation of coconut germplasm in the form of embryo cultures. This method also avoids the formalities of quarantine regulations which include treatment of the nuts with insecticide, fungicide and fumigation. Further, the embryo collection also reduces greatly the cost of transport. A simple protocol of culturing coconut embryos of 8 to 11 ' nuts was standardized at CPCRI and successfully utilized in the recent germplasm expeditions of the Institute. This protocol broadly consisted of four components *viz.* field collection of embryos, *in vitro* active storage, *in vitro* retrieval, and *ex vitro* establishment of plantlets.

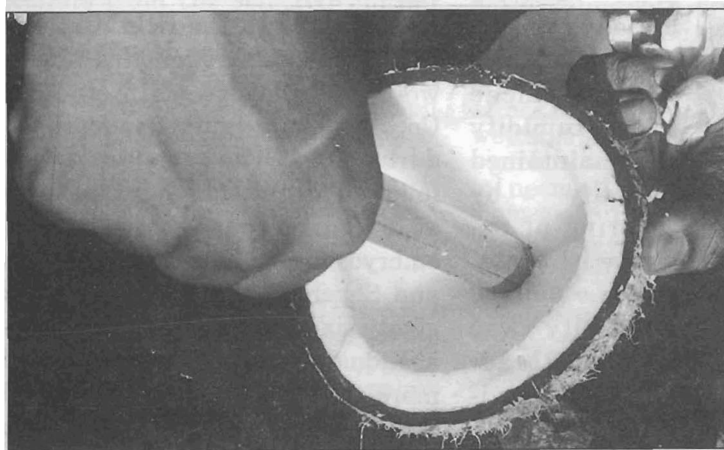

Fig. 1 : Collection of embryo

Field Collection

Simple and portable equipment is used for collection and direct inoculation of embryos in the field. First, the embryo along with a portion of the endosperm is scooped out by means of a cork borer from the dehusked and split-opened nut (Fig 1&2). The extracted embryo alone is collected in a beaker containing sterile water. The surface-sterilization of embryos in the field is done by 50% chlorine water for 20 minutes and washing 4-5 times in sterile water till the traces of chlorine is removed. The entire operation is done inside the portable, folding type plexiglass inoculation hood kept in open air. The surface of the hood is wiped thoroughly with absolute alcohol for disinfecting the surface. The embryos are then directly inoculated into a small screw cap bottle containing 5 ml of sterile water (*Fig 3*).

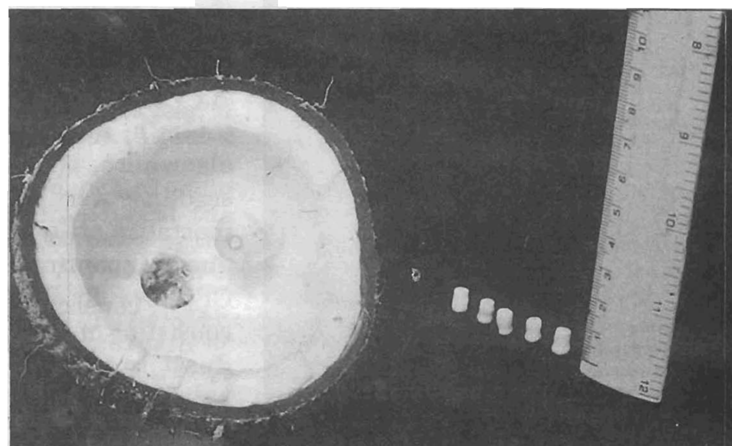

Fig. 2

In vitro active conservation

In vitro active conservation (short-term storage) of coconut zygotic embryos becomes necessary when the collection sites are located in far-off places. At CPCRI we observed that zygotic embryos could be stored in sterile water for 2 months. Compared to nutrient media, contamination of cultures was least in

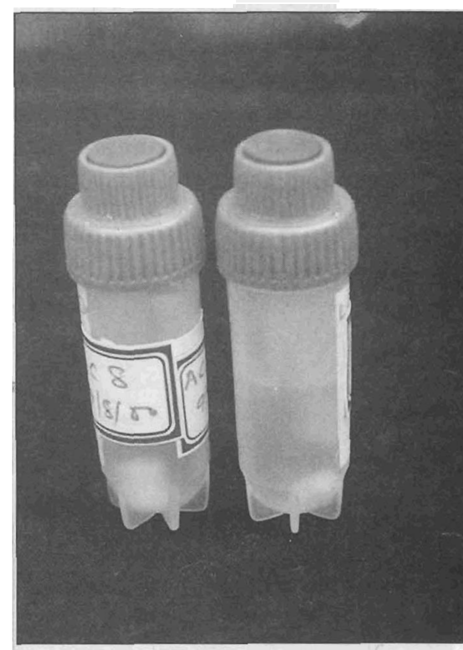

Fig. 3 : Collected embryos in a screw cap bottle containing sterile water

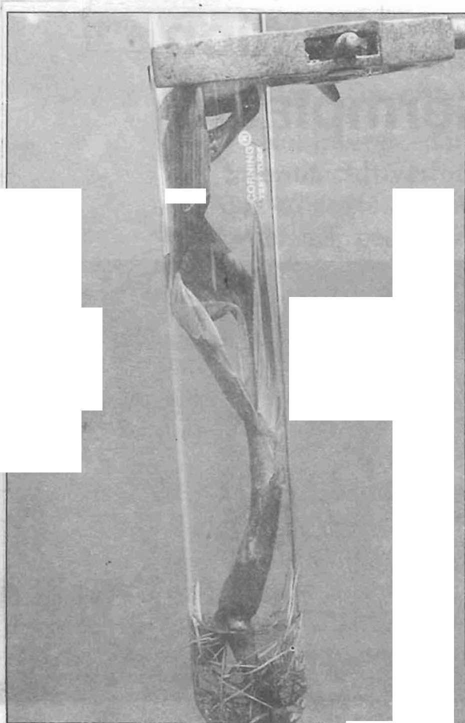

Fig. 4 : In vitro germination of coconut

sterile water. About 80% of the stored embryos (in sterile water) germinated when cultured in retrieval medium. Germination dropped to 13% when stored for 6 months. Embryos can also be stored for 2 months in sterile coconut water.

In vtiro retrieval

The basal medium used for retrieval of the embryos is Eeuwens' Y3. Solid medium is used till germination of the embryos and then shifted to liquid medium. The medium is supplemented with 30 g/ litre of sucrose for tall types and 60 g/ litre for dwarfs. Lack of adequate rhizogenesis was one of the main problems noticed at *in vitro* culture. Transferring the cultures to liquid medium supplemented with IBA (5 mg/ litre) and NAA (1 mg/ litre) enhanced root formation(*Fig. 4*)

Ex vitro studies

In a number of experiments elsewhere, the *in vitro* retrieved seedlings were reported to have high mortality on transplantation to soil due to poor root development. The CPCRI protocol uses a potting mixture consisting of sterile soil, sand and coir dust in equal proportions. As a precautionary measure before transplanting to the pots, the seedlings were treated with Bavistin (1 g/ litre) and thereafter with IBA solution (1000 ppm) for one hour each. The leaves were trimmed before transplanting to reduce transpiration. Higher humidity condition was maintained initially by covering plantlets with polythene bags. Humidity was maintained initially by covering plantlets with polythene bags. Humidity was reduced gradually by providing perforations to the polythene bag and later lifting the bag during night time and thereafter completely. Macro Y3 solution was given to the plantlets once in a month. The establishment of the plantlets (*Fig. 5*) in the pots, polybags and field was found to be very satisfactory and cent percent establishment was observed.

Fig. 5 :Cultured plantlets in poly bags

Application

The protocol for embryo culture developed by CPCRI was successfully used in two germplasm expeditions. The first was the collection and transportation of 87 embryos of six Pacific Ocean accessions maintained at the World Coconut Germplasm Centre (CPCRI), Andaman Islands, India. The embryos were retrieved *in vitro* at Kasaragod and successfully transplanted to the field. Out of 83 plantlets retrieved twentyfive plantlets were field planted at International Coconut Gene Bank for South Asia during 1996. The same protocol was followed for the subsequent collection of 15 exotic coconut germplasm (1242 embryos) from Mauritius, Madagascar and Seychelles and the first set of eighty six plantlets were field planted at Kidu during 1999 (*Fig. 6*). Recently more than 1500 embryos were field collected from Maldives, Comoros and re Union Islands.

Fig. 6 : Seedlings planted in a field