Short-term storage of coconut zygotic embryos in sterile water

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Coconut zygotic embryos (cultivar West Coast Tall) can be stored for two months in sterile water, Eeuwens Y3 media without charcoal or Y3 media without sucrose. When the embryos were transferred to the retrieval media, respectively 80.0, 66.7 and 66.7% germination were observed. This is the first report of the use of sterile water as the storage medium for coconut embryos.

EXCHANGE of coconut germplasm between different countries as well as from distant places within the same country is often beset with problems arising from the large nut size, short duration of dormancy and phytosanitary regulations. These difficulties can be largely overcome if the embryos alone can be scooped out, stored in aseptic conditions and germinated later *in vitro*. Karun *et al.*[1] described a simple procedure for direct field collection of 8- to 11-month old coconut embryos. The embryos thus collected were retrieved *in vitro* after keeping them at room temperature (29 ± 2°C) for 3–5 days in Eeuwens Y3[2] liquid medium[1]. However, immediate transportation of field collected embryos may not be possible when the collecting sites are located in distant places. This necessitates the short-term storage of embryos for 2 to 6 months before culturing in the nutrient medium. Germination of embryos and their further development depended greatly on the levels of activated charcoal and sucrose[3–5]. Hence, the absence of either activated charcoal or sucrose can inhibit the germination of embryos and their further growth. Assy-Bah *et al.*[5] reported that embryos of Dwarf type coconut can be stored for 6 months in MS medium with 2 gl^{-1} activated charcoal but without sucrose. However, use of nutrient medium for storing embryos has a higher risk of contamination. The use of sterile water as a storage medium is advantageous in this context and is reported for the first time here.

Besides sterile water (S3), two nutrient media viz., Eeuwen's Y3 medium without charcoal (S1) and Y3 medium without sucrose (S2) were evaluated for storing coconut embryos of tall type (West Coast Tall) for a short duration. As we wanted to collect a large number of embryos per accession, inclusion of immature embryos may become necessary and hence both mature (11-month old) and immature (8-month old) embryos were used in the present study. Embryos less than 8-month old age are difficult to extract because of their negligible size. Five embryos each of both mature and immature were inoculated in the aforesaid storage media and also in the retrieval medium (i.e. full Eeuwen's Y3) as control (S4). The pH was adjusted to 5.7 in all media. The experiment was replicated thrice. The inoculated embryos were stored in screw-capped vials containing 10 ml of medium sealed with parafilm and kept at room temperature (30 ± 2°C) for two months. The embryos were kept on the surface of the medium using filter paper bridge throughout the experiment.

Figure 1. Coconut embryos after two months of storage. Note the germination in S4. M, mature; I, immature.

It was observed that embryos stored in sterile water (S3) had light browning, and swelling in those stored in S1 (medium without charcoal). The swelling may be due to the absorption of water from the media to keep up osmolarity of the intracellular fluid and the formation of toxic metabolites in the embryos. Embryos kept in the retrieval medium (S4) germinated at the end of two months (Figure 1). The emergence of plumule from the embryonic axis was taken as evidence for germination.

The percentage germination of embryos after two months of transferring into the retrieval medium is shown in Table 1. The germination of mature embryos stored in different media is comparable and on par with that observed in retrieval media (S4) without any storage. In the previous study[1] too, the germination obtained was between 70% and 89%, under controlled conditions (temperature (27 ± 2°C) and humidity (55–60%)) and with periodical subculturing after every 21 to 25 days. It may therefore be concluded that the embryos had not lost the ability to germinate even after their storage for two months in all the three media considered in this study.

Table 1. Germination (%) of stored embryos after transferring into the retrieval medium

Category	S1	S2	S3	S4	Mean
Mature	66.67	66.67	80.00	66.67	70.00
Immature	20.00	6.67	0.00	20.00	11.67
Mean	43.33	36.67	40.00	43.33	

No storage in case of S4; observations were made after 2 months of inoculation into the retrieval medium. The germination significantly differs between mature and immature embryos but not among media and also there is no interaction effect. The S.E. plot with regard to values after angular transformation is 17.54.

The germination of immature embryos after two months of storage was negligible (Table 1). In S4 too, their performance was not satisfactory when compared to the earlier results under controlled conditions (58 to 84%)[1].

The present results clearly show that coconut embryos can be stored for two months in sterile water alone. This finding has far-reaching applications for sterile water is easily available and chances of contamination will be minimal compared to nutrient media. Further, the development of embryos will be arrested/negligible in sterile water compared to nutrient media and hence the effect of toxic substances at a later stage will also be minimal.

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