Table 1. *In vitro* response of mature embryos of barley on MS and B5 media with 2,4-D + kinetin

Medium	Embryos plated on medium containing 2,4-D (2 5 mg/l) + kinetin (0 5 mg/l)	Embryos producing callus	Calli producing green shoots on medium with lower 2,4-D (0 5 mg/l) + kinetin (0 2 mg/l)	Total no of green shoots produced in three subcultures on 2,4-D (0 5 mg/l) + kinetin (0 2 mg/l)
MS	22	20	20	456
B5	20	20	20	480

microtillering. Proliferation of shoots occurred without any intervening callus through proliferation of axillary buds by a process called microtillering. There was no marked difference between MS and B5 media with regard to shoot formation. In both the media, after 35 days, 8–10 shoots were formed. All these shoots were green and no albino shoots were observed (Figure 1). The regenerated shoots were rooted both on basal MS and B5 media. Rooting was better on basal B5 medium compared to MS. The rooted shoots were transferred to pots in the field conditions, directly without any hardening treatment in the months of October and November, after which they matured and set seeds.

The cultured mature embryos induced 3–5 shoots in 7–10 days with intervening callus on both MS and B5 media containing 2.5 mg/l 2,4-D and 0.5 mg/l kinetin (Figures 2 and 6). Callus was creamish-yellow, friable, and non-embryogenic in nature. There was no marked difference in the morphology of shoot-forming callus and non-shoot forming callus. Callus and shoots were separated and the shoots were kept on both basal MS and B5 medium for rooting. The separated calli were grown on a medium containing low levels of 2,4-D (0.5 mg/l) and kinetin (0.2 mg/l) under 16 h photoperiod (1400 Lux). Under these conditions the callus continued to produce multiple shoots up to 3–5 subcultures after which callus growth and differentiation ceased (Figure 3). The number of shoots (more than 30) produced was maximum in the third and the fourth subcultures. However, the callus under any condition could not be maintained or induced for further growth. No albino-shoot was observed in any of the cultures. The difference in percentage response and shoot morphogenesis between MS and B5 media was marginal (Table 1). However, rooting was better on basal B5 (Figure 4) medium in comparison to MS medium. The plantlets were transferred to pots under field conditions where they survived and set seeds (Figure 5). A 15-day pretreatment of regenerated shoots prior to field transfer with 2 mg/l IBA as reported by Bhattacharya[18] was not necessary in our work.

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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.

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.

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

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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Fatty acid profile of a marine *Nostoc calcicola* under saline and non-saline conditions

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A marine cyanobacterium *Nostoc calcicola*, when grown under non-saline condition synthesized quantitatively lower levels of fatty acids, except stearic acid. Salinity induced *de novo* synthesis of three unidentified fatty acids.

MANY cyanobacteria show adaptation to salinity and several physiological mechanisms underlying such adaptation have been identified[1–6]. In this communication, we report on the fatty acid profiles of a marine cyanobacterium grown in the absence and presence of salinity.

A marine cyanobacterium *Nostoc calcicola* BDU 40302 was obtained from the National Facility for Marine Cyanobacteria, Bharathidasan University, Trichy. The alga was grown photoautotrophically at 28 ± 1°C with a 12 h light (3000 lux): dark regimen in nitrogen-free artificial seawater ASN III medium[7] with 25 ppt NaCl. For the salinity-shift experiments, cultures

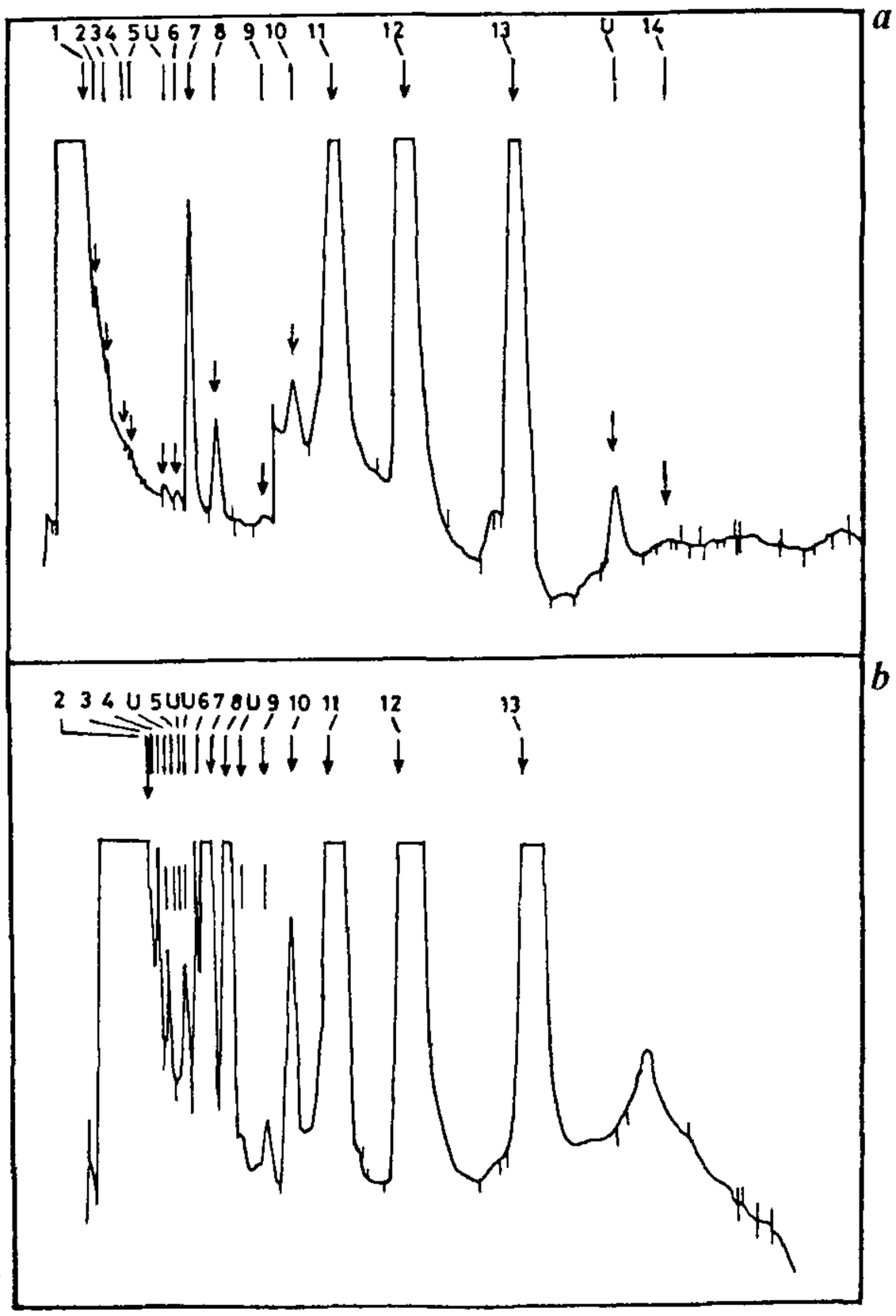

Figure 1. Fatty acid profile of a marine *Nostoc calcicola* grown in the absence (*a*) and presence (*b*) of NaCl (25 ppt) 1, undecanoic acid, 2, lauric acid, 3, tridecanoic acid, 4, myristic acid, 5, pentadecanoic acid, 6, palmitic acid, 7, palmitoleic acid, 8, heptadecanoic acid, 9, stearic acid, 10, oleic acid, 11, nonadecanoic acid, 12, arachidic acid, 13, henecosanoic acid, 14, behenic acid (U, unidentified fatty acids).