370, Chittimutyalu and Gopal Bhog were grown to maturity (figure 1C) and the seeds collected. The Tc2 seedlings of these varieties were subjected to 0.75% NaCl (electrical conductivity 13.5 mmhos) throughout the life cycle (electrical conductivity was checked every alternate day). During early stages of growth, the plants under stress showed certain changes in morphology compared to control plants. The leaves were generally narrow under salt stress.

The present studies clearly suggest the genotype dependence of salt tolerance. The experiments involving use of seawater for selection of salt-tolerant lines provide new information and may ultimately lead to the exploitation of seawater directly in raising rice crop.

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IN VITRO REGENERATION, FIELD TRANSFER OF PLANTLETS AND GROWTH TO MATURITY OF PLANTS OF *SORGHUM BICOLOR* (L.) MOENCH

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SORGHUM is an agronomically important crop and is used as food, fibre, fodder and fuel[1]. Tissue culture propagation techniques have been tried, with some success. Plant regeneration from callus derived from tillering nodes[2], mature embryos[3], immature embryos[4,5], leaf[6] and inflorescence segments[7] has been reported. The present report describes plant regeneration in *Sorghum bicolor* via somatic embryogenesis as well as through shoot-bud formation and the transfer of plantlets to field for maturity and seed set.

Grains of *Sorghum bicolor* (L.) Moench cv. CSH-5 obtained from the Agriculture Research Station, Durgapura, were sown in plots in the University Botanical Garden. Tassels with immature embryos at milk stage were surface-sterilized with 0.1% $HgCl_2$ solution for 3–5 min and washed thrice with sterile water. Immature embryos were dissected aseptically in a clean-air hood, and cultured on Murashige and Skoog (MS)[8] medium supplemented with auxins and cytokinins and solidified with 0.8% agar. The pH of the medium was adjusted to 5.8 before autoclaving. All cultures were incubated at 26 ± 2°C under continuous illumination. All experiments were repeated thrice.

Callus from the cultured immature embryos was initiated on MS medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D, 1 mg/l) in combination with zeatin (Z, 2.2 mg/l) or kinetin (K, 0.2 mg/l) or coconut water (CW, 10% v/v). Callus initiation started after 10 days of culture and in five weeks a good green organogenic callus was obtained. A number of small green protuberances were visible on the surface of the callus, from which shoot buds differentiated and eventually formed shoots. Embryoid formation was also observed in some cultures, as shown in table 1 and figure 1a, b. Callus formed on media with 2,4-D (1–10 mg/l) was compact, yellow, hard in texture and slow-growing. Callus formed on different media was maintained by subculturing every 3–4 weeks on MS medium with 2,4-D (2.5 mg/l) + K (0.5 mg/l).

Table 1 *Shoot and embryoid differentiation from cultured immature embryos of Sorghum bicolor*

Supplement	Per cent cultures showing		
	Callus formation	Shoot buds	Embryoids
2, 4-D (1 mg/l)	78.0 ± 2.0	—	—
2, 4-D (3 mg/l)	76.25 ± 11.2	—	—
2, 4-D (1 mg/l) + K (0.2)	73.33 ± 12.01	—	21.66 ± 7.60
2, 4-D (3 mg/l) + K (0.2)	72.85 ± 12.85	—	15.71 ± 4.28
2, 4-D (1 mg/l) + CW (10%)	81.1 ± 6.7	22.33 ± 6.7	25.66 ± 3.47

– represents no response.

Numerous shoots along with roots were obtained in 4–5 weeks from the subcultured callus on MS medium with K (3 mg/l) + IAA (0.5 mg/l) or IBA (0.2 mg/l). A second subculture was required for

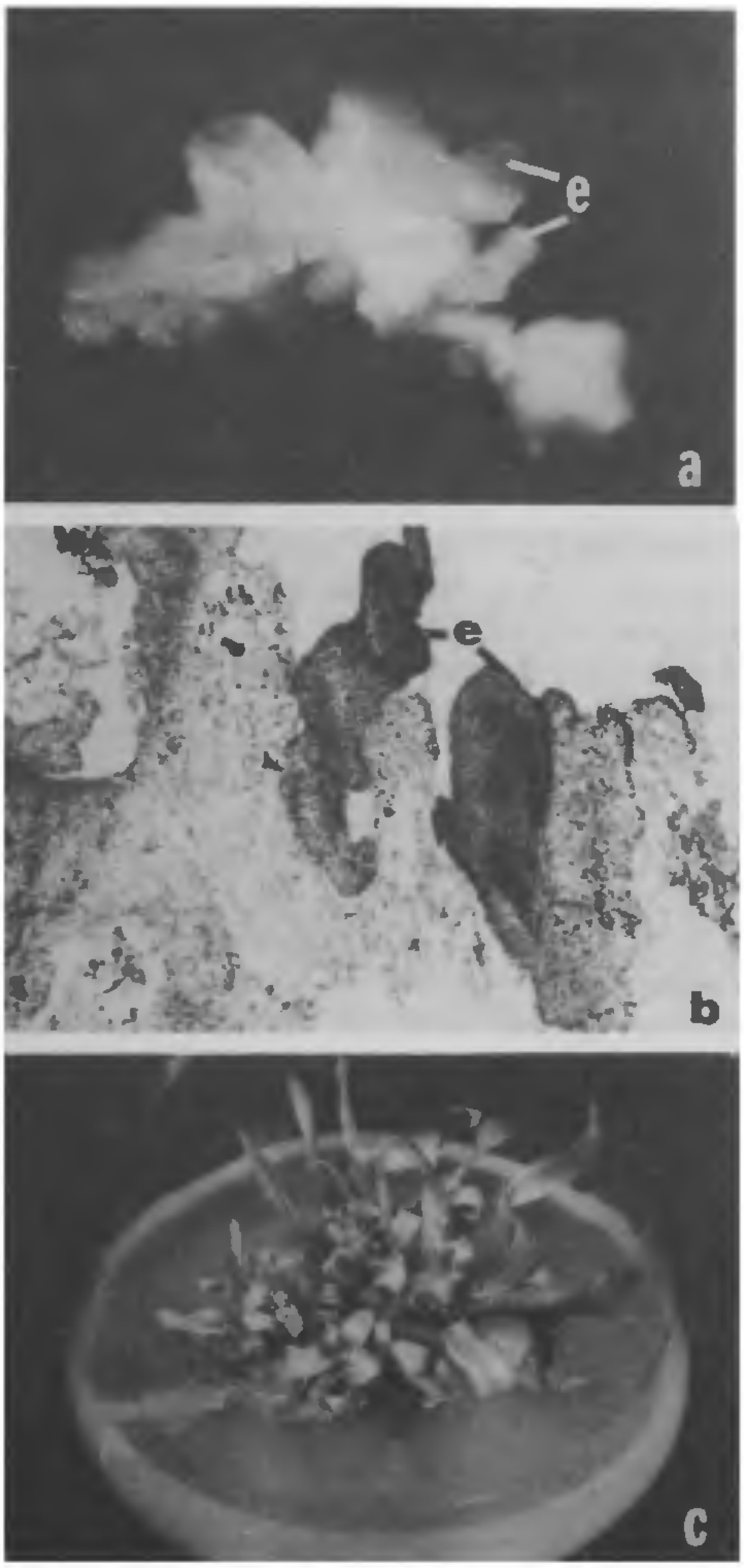

Figure 1a–c. **a.** Isolated embryoids (e) differentiated on MS with 2,4-D (1 mg/l) + CW (10%) (× 35); **b.** Section of embryogenic callus exhibiting developing embryoids (e) (× 40), and **c.** Shoot differentiation on MS + K (3 mg/l) + IAA (0.5 mg/l).

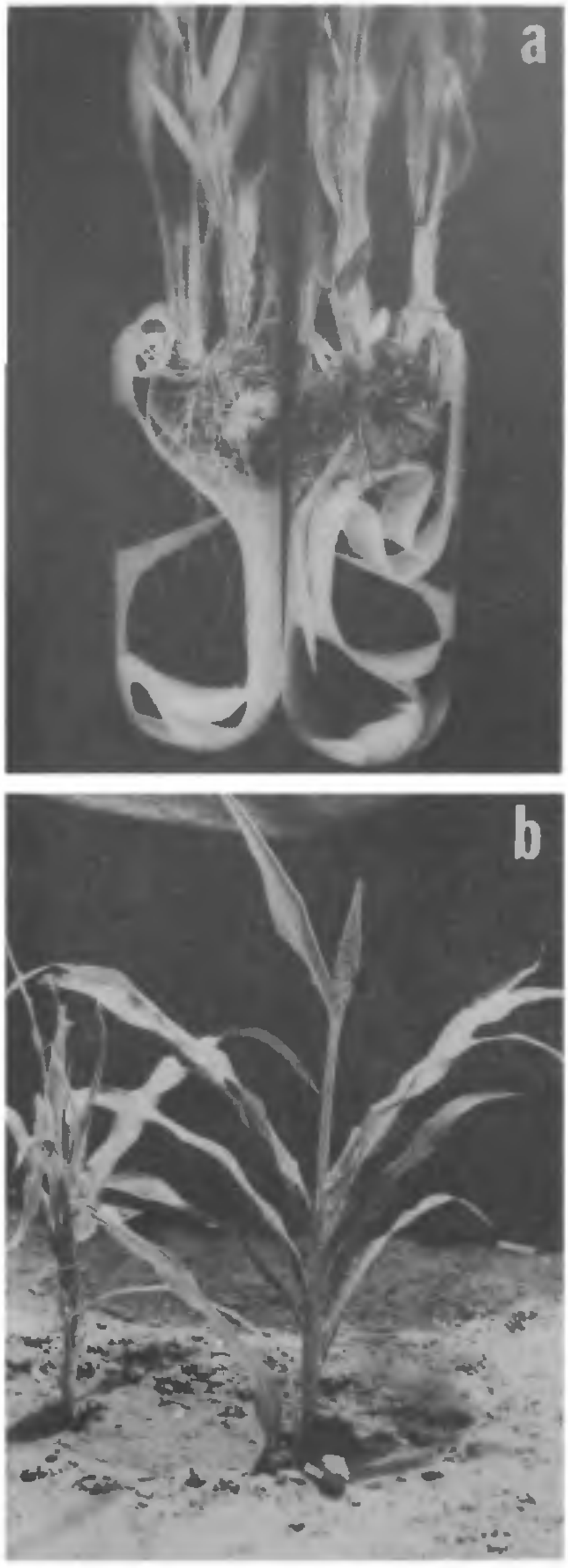

Figure 2a, b. **a.** Rooting from *in vitro* shoots on MS + NAA (5 mg/l), and **b.** Plants transferred to field.

tillering and elongation of shoots (4–5 cm long) (figure 1c).

The callus containing embryoids was transferred to a medium with K (3 mg/l) + IAA (0.5 mg/l), where it formed plantlets in 5 weeks.

The initial callus formed on media with both auxin and cytokinin showed higher frequency of regeneration compared to the callus formed on a medium containing auxin alone. Regeneration was also obtained on other media containing 2,4-D (1 mg/l) + K (0.2 mg/l) or BAP (0.2 mg/l) + NAA (1 mg/l) or NAA (5 mg/l). But in all these media the number of regenerated shoots was low.

For rooting, the *in vitro* shoots were isolated and transferred to filter paper bridges in test tubes with liquid MS medium or to agar media containing IAA (5 mg/l), IBA (3 mg/l), NAA (5 mg/l), or MS medium without growth regulators. The percentage of shoots forming roots on the above media was 71, 77, 84 and 25 respectively. Although roots were formed on all these media, in terms of frequency, number and type of roots formed, the best media were those containing NAA (5 mg/l) or IBA (3 mg/l). Roots emerged directly from the base of shoots 2 weeks after transfer, resulting in the establishment of complete plantlets (figure 2a). It was observed that for best rooting the length of shoots should be about 4–5 cm. Plantlets thus formed were transferred to pots containing a 3:1 mixture of sterilized garden soil and vermiculite. Pots were kept covered with a glass jar in the culture chamber. After 12–15 days of acclimatization in the growth room at 26 ± 2°C, the plants were transferred to soil and maintained to maturity. Plants flowered and set seeds normally (figure 2b). Seeds were slightly shrunken but viable. Immature embryos from the regenerated plants were also cultured and could be regenerated.

In the present report plant regeneration was observed through embryogenesis as well as through shoot-bud formation, in contrast to a previous report which describes only embryogenesis[3]. Another important observation is that callus formed from immature embryos on medium containing auxin in combination with cytokinin was compact, nodulated, green and highly regenerating, whereas callus initiated on medium containing only auxin was yellow, hard and less regenerative. This has also been reported for callus formed from immature and mature embryos[3] and leaves[6] of grain *Sorghum*. A second subculture was necessary for good tillering and elongation of shoots (4–5 cm long).

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EFFECT OF AESTIVATION ON FOOD UTILIZATION IN THE FRESHWATER SNAIL *PILA GLOBOSA* (SWAINSON)

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OUR previous publications concerned with the effects of food quantity[1–3], quality[4,5], crowding[6], water level[6] and starvation[7,8] on food utilization in the freshwater snails. The present paper reports the effect of aestivation on energy budget in the freshwater snail *Pila globosa*.

Snails of *P. globosa* were collected from pond Idumban (Palni, Tamil Nadu) and acclimated to laboratory conditions. Snails of 12 ± 2 g live weight were recruited from the stock and buried in mud troughs at 7.5 cm depth (temperature: 32°C; moisture: 8%) and forced to aestivate for 5 months. Fifty aestivated snails were selected and fed on *Chara fragilis* at chosen feeding levels (10 to 100%) for one month period. Every day a control sample of 5 g