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IN VITRO SELECTION FOR SALINITY AND REGENERATION OF PLANTS IN RICE

K. SUBHASHINI and G. M. REDDY

Department of Genetics, Osmania University,
Hyderabad 500 007, India.

SOIL salinity affects nearly one-third of the irrigated land in the world and hence the development of rice varieties with greater tolerance to salinity may lead to use of more area for rice cultivation and to increased productivity of rice in saline lands. Tissue culture techniques are being exploited increasingly in the selection of salt-tolerant lines in rice¹. Callus tissues of higher plants have been used in the selection of desired mutant cell lines². The variants are generally selected by subjecting callus tissues to selection pressure with the appropriate substance¹. Lines tolerant to NaCl have been isolated in alfalfa³ and tobacco⁴. Earlier studies with rice have led to the selection and regeneration of 1% NaCl-tolerant lines of two cultivars Jaya and Tellahamsa⁵. The present study mainly deals with the screening of five genotypes for salt tolerance in callus cultures not only with NaCl but also with different concentrations of seawater directly, and the regeneration of plants from these salt-tolerant callus cultures.

Mature seeds of rice cultivars Basmati 370, Pakistani Basmati, Chittimutyalu, Gopal Bhog and Randhunipagalu were dehusked, sterilized with 0.1% mercuric chloride for 7 min, washed thoroughly with sterile distilled water, and pre-soaked for 24 h. Embryos were separated and cultured on Linsmaier and Skoog⁶ (LS) medium supplemented with 2 mg/l of 2,4-dichlorophenoxyacetic acid (2,4-D). All the cultures were kept under continuous white fluorescent light and maintained for 3 weeks at $26 \pm 1^\circ\text{C}$ to induce callus. Profusely growing calli were inoculated onto LS selection media containing 1, 2 or 3% NaCl, or 25, 50 or 75% seawater with 2 mg/l 2,4-D.

There was a decrease in callus growth with increase in salt concentration in the medium. Calli transferred to the NaCl or seawater selection medium usually turned brown, indicating necrosis of the calli. However, portions of the calli grew (figure 1A). These were subcultured onto fresh salt/seawater selection media. The selection process was repeated thrice (60 days) to obtain vigorously growing salt-tolerant calli. In 1 and 2% NaCl and 25 and 50% seawater, 40% of the calli were necrotic. In 3% NaCl and 75% seawater, 95% of the calli were necrotic.

The tolerant calli were then transferred to regeneration LS medium with 4 mg/l kinetin and 1 mg/l IAA. High frequency of regeneration (21–60%) was observed in all the genotypes on medium without

Table 1 Regeneration of whole plants from salt-tolerant calli in different cultivars of rice

Cultivar	Control	Frequency of plant regeneration (%)			
		NaCl* (%)		Seawater* (%)	
		1	2	25	50
Basmati 370	50–55 (15)	21–23 (6)	—	28–30 (7)	19–20 (4)
Chittimutyalu	56–60 (17)	23–25 (6)	3 (1)	30–32 (9)	16–18 (5)
Gopal Bhog	60 (18)	25–26 (7)	—	37–38 (12)	10–13 (4)
Pakistani Basmati	25–30 (8)	13–16 (4)	—	18–19 (4)	8 (3)
Randhunipagalu	21 (6)	5 (2)	—	7 (2)	—

*NaCl and seawater at given concentrations were present in callus selection medium and absent in regeneration medium. Average of 30 cultures/treatment/genotype; Figures in parentheses are numbers of regenerated plants.

NaCl/seawater (table 1). The frequency of regeneration decreased with increasing concentration of NaCl/seawater in all the genotypes. Based on regeneration ability (table 1), the cultivar Randhuni-pagalu was more sensitive to salt. A total of 76 salt-tolerant plants of the five cultivars were regenerated from calli and transferred to pots (table 1). The

differences in the response of these varieties to NaCl/seawater may be genotype-dependent. It may be suggested that sodium chloride decreases callus growth probably by decreasing the rate of cell division and hence retaining the regeneration capacity.

The Tc1 (tissue culture raised) plants of Basmati

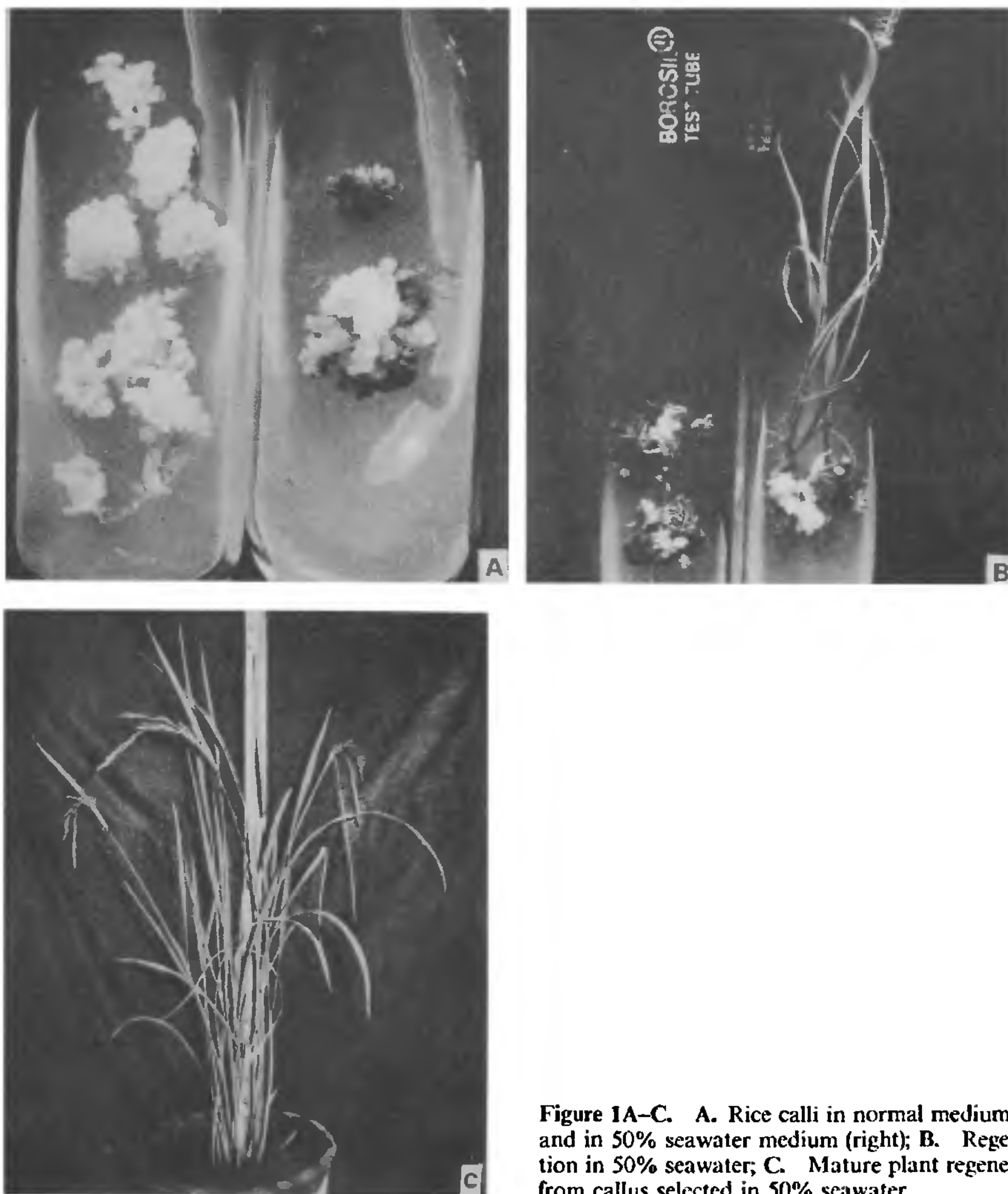


Figure 1A-C. A. Rice calli in normal medium (left) and in 50% seawater medium (right); B. Regeneration in 50% seawater; C. Mature plant regenerated from callus selected in 50% seawater.

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

VANDANA SHARMA, S. L. KOTHARI and N. CHANDRA

Department of Botany, University of Rajasthan, Jaipur 302 004, India.

SORGHUM is an agronomically important crop and is used as food, fibre, fodder and fuel¹. Tissue culture propagation techniques have been tried, with some success. Plant regeneration from callus derived from tillering nodes², mature embryos³, immature

embryos^{4,5}, leaf⁶ and inflorescence segments⁷ 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% HgCl₂ 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.