

# In Vitro Propagation of *Salicornia bigelovii* by Shoot-tip Cultures

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*Salicornia bigelovii* Torr., an annual, succulent species of Chenopodiaceae, has the potential to become one of the first seawater-irrigated crop plants (Glenn et al., 1991). This plant was identified as having the greatest promise among many halophyte species tested for possible domestication as a new oilseed crop (O'Leary, 1988; O'Leary et al., 1985). The objective of this study was to develop a tissue culture medium for clonal propagation of selected plants.

*Salicornia* seeds were germinated in a greenhouse in 1.8-liter plastic pots containing a mixture of 1 peat : 1 vermiculite : 1 perlite (by volume) and were irrigated with fresh water. Plants were fertilized weekly with a nutrient solution containing 200 ppm N, 88 ppm P, and 166 ppm K. Shoot tips (8 to 10 mm long) of 2-month-old plants were excised and surface-sterilized for 10 min in 0.26% sodium hypochlorite containing 5 mg Tween 20 (surfactant)/liter, followed by four rinses in sterile distilled water. The basal ends of the tips were recut and cultured on a medium containing MS salts (Murashige and Skoog, 1962), B-5 vitamins (Gamborg et al., 1968), and (in mg-liter<sup>-1</sup>) 100 inositol, 2 glycine, 30,000 sucrose, and 10,000 Difco Bacto-agar. The combinations of 0.54  $\mu$ M and 10.74  $\mu$ M 1-naphthaleneacetic acid (NAA) and 0.44  $\mu$ M and 8.89  $\mu$ M N-(phenylmethyl)-1 *H*-purine-6-amine (BA) were used to study their influence on shoot multiplication and root development.

Cultures were grown on 20 ml medium contained in 25 × 250-mm glass tubes. Each treatment had 20 replications and the experiment was repeated. All cultures were grown at 21 ± 2°C under a 30 to 46  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup> light intensity produced by cool-white fluorescent lamps for 16 h·day<sup>-1</sup>.

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The number of shoots and roots developed on the initial culture varied with the growth regulator concentration. Explants cultured for 8 weeks on a medium containing 0.54  $\mu$ M NAA and 8.89  $\mu$ M BA produced the most shoots per culture (Table 1). The mean length of new shoots was inversely related to the mean number of shoots obtained on the three growth regulator combinations (Table 1). Shoot tips cultured on medium containing 10.74  $\mu$ M NAA and 0.44  $\mu$ M BA initiated an average of 12 roots/explant; cultures did not produce roots on media containing 8.98  $\mu$ M BA. All growth regulator treatments induced callus formation at the base of the explants; the callus did not interfere with either shoot proliferation or root generation.

Shoots, when divided and subcultured on the MS basal medium supplemented with 0.54  $\mu$ M NAA and 8.89  $\mu$ M BA, continuously produced 12 to 30 new shoots per culture every 8 weeks (Fig. 1a). The individual shoots elongated and produced healthy roots when transferred to a medium containing 10.74  $\mu$ M NAA and 0.44  $\mu$ M BA (Fig. 1b). The rooted plantlets were transplanted into 0.6-liter plastic pots containing a mixture of 1 peat : 1 vermiculite : 1 perlite. These plants were kept inside a humid polyethylene tent in the greenhouse and acclimatized for 10 days by gradually opening the plastic cover. Most plants survived and grew like seed-grown plants. Our results indicate that mass propagation of *salicornia* clones from shoot-tip cultures is possible and that this technique may be useful in breeding programs.

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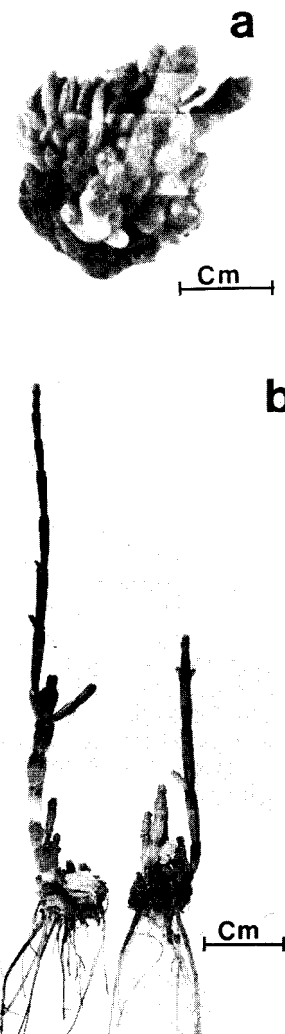


Fig. 1. In vitro culture of *Salicornia bigelovii* shoots: (a) shoot proliferation on MS medium supplemented with 0.54  $\mu$ M NAA and 8.89  $\mu$ M BA after 8 weeks; (b) root generation on MS medium containing 10.74  $\mu$ M NAA and 0.44  $\mu$ M BA after 8 weeks.

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Table 1. Shoot multiplication and root formation from *Salicornia bigelovii* shoot tips cultured in vitro for 8 weeks on media containing NAA and BA.

Growth regulator ( $\mu$ M)		Shoots			Roots (no.)/culture $\pm$ SE
NAA	BA	No. $\pm$ SE	Length (mm) $\pm$ SE		
10.74	0.44	6.2 $\pm$ 2.2	37.4 $\pm$ 29.0		12.0 $\pm$ 6.5
10.74	8.89	16.4 $\pm$ 3.4	16.8 $\pm$ 4.4		0.0 $\pm$ 0.0
0.54	8.89	23.0 $\pm$ 8.0	10.8 $\pm$ 4.1		0.0 $\pm$ 0.0