Somatic Embryogenesis in Members of the Plumbaginaceae Ornamental *Statice Limonium* and Sea Thrift *Armeria maritima*

Mohammed A.M. Aly¹, Bala Rathinasabapathi², and Sheevani Bhalsod
Horticultural Sciences Department, University of Florida, Gainesville,
FL 32611-0690

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Abstract. Many members of the Plumbaginaceae are important flower crops wherein propagation is hindered by poor seed germination. Micropropagation via organogenesis is commercially practiced for certain *Limonium* species. However, somatic embryogenesis was not reported for members of the Plumbaginaceae until recently for *L. bellidifolium* Durmort. The induction of somatic embryogenesis from cotyledon explants in a modified Murashige and Skoog (MS) medium was examined in four other members of this family, *Limonium aureum* O. Kuntze, *L. latifolium* O. Kuntze, *L. sinuatum* Mill., and *Armeria maritima* Willd. Induction of embryogenic callus was achieved in all the species examined on MS medium supplemented with 4.5 μM 2,4-D and 88 or 118 mM sucrose. Species of the genus *Limonium* responded better than *A. maritima* Willd. in somatic embryo induction and maturation. Somatic embryos of *L. aureum* O. Kuntze matured readily on MS medium supplemented with 0.93 μM kinetin and 88 mM mannitol. Chemical name used: 2,4-Dichlorophenoxy acetic acid (2,4-D).

Members of the Plumbaginaceae, genus *Limonium*, are of commercial importance in ornamental horticulture for fresh and dry cut flowers (Harada, 1992). Tissue culture techniques have been applied only to a limited extent in the Plumbaginaceae. Shoot regeneration via organogenesis was achieved in *L. perezii* F.T. Hubb. (Kunitake and Mii, 1990), *L. perigrinum* R.A. Dyer (Seelye et al., 1994), and *L. altaica* Mill. (Jeong et al., 2001). We have recently developed a rapid and efficient protocol to induce somatic embryogenesis in *L. bellidifolium* (Aly et al., 2002). This protocol employed relatively low levels of 2,4-D in MS (Murashige and Skoog, 1962)-based medium to induce embryogenic callus from cotyledon explants and MS-based medium with kinetin for somatic embryo maturation. The objective of the current study is to test the efficacy of that protocol to induce embryogenesis in four other members of the Plumbaginaceae, *L. aureum* O. Kuntze, *L. latifolium* O. Kuntze, *L. sinuatum* Mill., and *Armeria maritima* Willd.

Materials and Methods

Seed sterilization, germination and explant culture. Seeds of *Limonium aureum*, *Limonium latifolium*, *L. sinuatum* (cv. Soiree Rose) and *Armeria maritima* were purchased from Park Seed Co. (Greenwood, S.C.). Seeds were surface-sterilized and germinated as described (Aly et al., 2002). Cotyledon (2–3 mm long) explants from 5-day-old seedlings were cultured abaxial side facing up, 10 cotyledons per 100 $\times$ 15-mm petri plate, on MS medium containing B5 vitamins (Gamborg et al., 1968), 88 or 118 mM sucrose, 0.93 μM kinetin, and 4.5 μM 2,4-D, pH 5.8. For each species, treatments were done in triplicate with 30 explants per replication (n = 90). The plates were sealed with parafilm (American National Can, Chi-

cago) and incubated at 25 °C under 16-h day/8-h night light regime (PPF 46 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, cool-white fluorescent lamps).

Somatic embryo maturation and germination. For maturation, *L. aureum* somatic embryos at late-globular stage were subcultured on MS media containing 88 or 118 mM sucrose with or without 0.93 kinetin and with or without 88 mM mannitol. Each treatment had three 100 $\times$ 15 mm Petri plates, each with four callus pieces (n = 12) and the experiment was repeated twice. For germination, cotyledonary stage embryos were transferred to MS basal medium lacking sucrose, four per 100 $\times$ 15 mm Petri plate. Twenty of the germinated plantlets were transferred to sterile potting medium in Magenta™ vessels (3" $\times$ 3" $\times$ 4"; Sigma, St. Louis), irrigated with Hoagland's nutrient solution (Hoagland and Arnon, 1950), acclimatized by gradually lowering the humidity over 14 d and transferred to the greenhouse.

Statistical treatment. Experiments were set up following completely randomized design and analyses of data were performed using the SAS statistical package (SAS Institute, 2000). Following analysis of variance, grouped means *t* test and Duncan's multiple range test were used for mean comparisons.

Results and Discussion

Cotyledon explants from the four species initiated embryogenic callus within 10 d in culture, three species of *Limonium* producing more somatic embryogenic callus than *A. maritima* (Table 1). Induction frequencies at 118 mM sucrose was significantly less than that observed for 88 mM sucrose for *L. latifolium* and *A. maritima* and more for *L. sinuatum* (Table 1). However, there were many arrested embryos at 118 mM sucrose in all the three species (data not shown).

Upon transfer to the MS medium, about 25% of the transferred *L. aureum* late globular callus masses developed into cotyledonary stage somatic embryos (Table 2) and resulted in plantlets that could be transferred to soil at a frequency of 10 plantlets per every 12 callus pieces. Kinetin in MS medium significantly

Table 1. Response of *Limonium sinuatum*, *L. latifolium*, *L. aureum*, and *Armeria maritima* cotyledon explants to MS medium supplemented with 4.5 μM 2,4-D, 0.93 μM kinetin, and 88 or 118 mM sucrose. Explants were scored for the induction of embryogenic callus 30 d after culture.

Species	% Response (mean) ^z		Means ^y
	88 mM sucrose	118 mM sucrose	
<i>L. aureum</i>	56.7	ND ^x	ND
<i>L. latifolium</i>	57.2	36.1	*
<i>L. sinuatum</i>	79.0	88.0	**
<i>A. maritima</i>	28.6	22.4	*

^zMean are for % response from three experiments. In each experiment n = 30 per treatment.

^yMeans from the two sucrose levels for each species were compared using grouped means *t* test.

^xND = not determined.

^{*},**Significant at *P* = 0.05 or 0.01, respectively.

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¹Current address: Department of Genetics, Cairo University, Cairo, Egypt.

²To whom reprint requests should be addressed; phone 352/392-1928 xt 323;

E-mail brath@mail.ifas.ufl.edu

Table 2. Effect of kinetin and mannitol on the regeneration of cotyledonary stage plantlets from cultured late-globular somatic embryos of *Limonium aureum*. Uniform callus pieces (≈ 100 mg) were inoculated on the indicated medium and scored for regeneration after 4 weeks. Results are means from 12 replicates.

Maturation medium	% Germination ^z	Mean no. plantlets/callus ^y
MS medium	25	0.85 a
MS medium + 0.93 μ M kinetin	100	4.85 b
MS medium + 88 mM mannitol	16	0.31 a
MS medium + 0.93 μ M kinetin + 88 mM mannitol	100	6.77 c

^z(Number of germinated late globular embryos/total number of late globular embryos inoculated) $\times$ 100.

^yMeans indicated by same letters are not significantly different at $P = 0.005$ by Duncan's multiple range test.

increased both per cent germination and the number of plantlets recovered per callus (Table 2). Addition of both kinetin and mannitol resulted in significantly more plantlets recovered than MS medium alone or with only kinetin or mannitol (Table 2). This suggests that kinetin in the germination medium is important for inducing differentiation of embryos. Mannitol, an inert osmoticum further enhanced the recovery of plantlets following germination of the somatic embryos. All of a sample of twenty *L. aureum* plantlets trans-

ferred to the soil established and flowered at the greenhouse.

Maturation of somatic embryos was observed infrequently for *L. latifolium* and not at all for *L. sinuatum* and *A. maritima* (data not shown). By employing an induction medium containing low levels of 2,4-D, embryogenic callus was induced in three species of ornamental statice and *A. maritima*. Maturation medium supplemented with kinetin and mannitol produced high-frequency plant regeneration in *L. aureum*.

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