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

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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 Durmorn. 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, L. 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).

Materials and Methods

Seed sterilization, germination and explant culture. Seeds of Limonium aureum, L. 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 × 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, Chicago) and incubated at 25 °C under 16-h day/8-h night light regime (PPF 46 µmol-m⁻²-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 × 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 × 15 mm Petri plate. Twenty of the germinated plantlets were transferred to sterile potting medium in Magenta™ vessels (3” × 3” × 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.

Results and Discussion

Cotyledon explants from the four species initiated embryogenic callus within 10 d in culture, three species of Limonium producing more 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

| Species          | 88 mM sucrose | 118 mM sucrose |
|------------------|---------------|----------------|
| L. aureum        | 56.7          | ND             |
| L. latifolium    | 57.2          | 36.1           |
| L. sinuatum      | 79.0          | 88.0           |
| A. maritima      | 28.6          | 22.4           |

*Mean are for % response from three experiments. In each experiment 88 and 118 mM sucrose, 0.93 µM kinetin, and 88 or 118 mM sucrose. Explants were scored for the induction of embryogenic callus 30 d after culture.

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

ND = not determined.

*Significant at P = 0.05 or 0.01, respectively.

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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                     | %  | Mean no. plantlets/callus |
|---------------------------------------|----|--------------------------|
| MS medium                             | 25 | 0.85 a                   |
| MS medium + 0.93 µM kinetin           | 100| 4.85 b                   |
| MS medium + 88 mM mannitol            | 16 | 0.31 a                   |
| MS medium + 0.93 µM kinetin + 88 mM mannitol | 100| 6.77 c                   |

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

\(^{y}\) Means 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 transferred 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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