In Vitro Propagation of *Coryphantha macromeris*

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Cacti are asexually propagated by offsets and cuttings or sexually via seeds (Johnson and Emino, 1979a). However, many species are slow-growing and do not produce offsets. Seeds are sometimes difficult to obtain (Mauseth, 1977) and seedlings are susceptible to damping-off (Mauseth, 1979). As one-fourth of all cacti native to the United States are rare or in danger of extinction (Benson, 1977), in vitro propagation of cacti is an attractive alternative for multiplication and maintenance of valuable germplasm.

Callus induction (Ault and Blackmon, 1987; Johnson and Emino, 1979a; Mauseth, 1977; Steinhart, 1962) and in vitro growth regulation (Mauseth and Halperin, 1975; Minocha and Mehra, 1974) have been investigated for some cacti species. Johnson and Emino (1979a, 1979b) reported successful in vitro propagation of *Mammillaria elongata* DC., *Opuntia polyacantha* Haw. P. and *Hylocereus calcaratus* (A. Berger) Britt & Rose. However, each cactus species required a different auxin: cytokinin balance for shoot induction (Johnson and Emino, 1979b). Ault and Blackmon (1987) reported in vitro shoot proliferation from *Ferocactus acanthodes* (Lemaire) Britt. & Rose. We describe an in vitro propagation method for *Coryphantha macromeris* (Engelm.) Lem. from callus culture.

Seeds were surface sterilized in 1.05% (w/v) NaOCl for 10 min, rinsed in sterile water, and then aseptically germinated on Murashige and Skoog (1962) (MS) inorganic salts, pH 5.9, and solidified with 6 g Bacto agar/liter. All cultures were maintained under a $24 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ light intensity (Sylvania Grow Lux wide spectrum fluorescent bulbs) for 16 h daily at $26 \pm 3^\circ\text{C}$.

Shoot explants from the aseptically grown seedlings were cultured on MS medium supplemented with (per liter) 0.4 mg thiamine-HCl, 100 i-inositol, 20 g sucrose, 44

μM *N*-(phenylmethyl)-1-*H*-purine-6-amine (BA), and 0.5 μM 2,4-dichlorophenoxyacetic acid (2,4-D) at pH 5.7 for callus proliferation. Callus grew in 3 to 4 weeks and was maintained through subculture on the identical medium. This callus has been subcultured for >4 years. Shoot formation occurred sporadically on this callus (Fig. 1A). For shoot multiplication, 6- to 8-mm-diameter callus pieces with an average weight of 0.41 g were subculture on identical medium without plant growth regulators. In 6 weeks the callus diameter had increased to 20 to 40 mm and averaged 4.45 g. Multiple shoots (Fig. 1B) developed after 6 to 8 weeks and as many as 20 shoots formed per culture tube. Shoot cultures have been subculture at 4- to 8-week intervals on medium free of plant growth regulators for >2 years.

Root initiation occurred on shoots in the multiplication medium at a low frequency (36/190, 19%). For enhanced rooting, shoots were separated and cultured on one-half strength MS inorganic salts plus (per liter) 0.4 mg thiamine-HCl, 20 g sucrose, and 1.5 g Gelrite (Schweizerhall, N.J.). Initial rooting experiments compared Gelrite-solidified medium to vermiculite moistened with the rooting medium (MS salts, thiamine-HCl and sucrose), both in 25×150 -mm culture tubes. Roots formed in 4 weeks but at a low frequency on the agar medium (4/20 shoots rooted). After 20 weeks, 90% of the shoots in both treatments (18/20 gelrite, and 36/40 vermiculite) had rooted. Rooted shoots were transferred to 40 ml of a 1 peat : 1 vermiculite (v/v) potting mixture contained in 55-ml plastic pots. Pots were placed in a plastic tray covered with plastic wrap for ≈ 2 weeks to gradually harden off the plants. The plants were kept in the culture room ($24 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ light intensity 16 h daily at $26 \pm 3^\circ\text{C}$) and 55% survived.

Rooting efficiency was examined in 25×150 -mm culture tubes containing 25 ml MS medium and in Magenta GA-7 vessels (Magenta Corp., Chicago) with 10 ml MS medium. Of shoots placed in test tubes, 64% (36/56) formed roots after 12 weeks and 90% after 20 weeks. Ten milliliters of medium that covered the bottom of Magenta vessels desiccated during the culture period of 8

Fig. 1. (A) Callus cultures of *C. macromeris* on the callus induction medium, 0.8 $\times$. Some shoot formation (arrows) occurred on this medium. (B) Multiple shoot development (arrows) from *C. macromeris* callus cultured on medium lacking plant growth regulators, 1.-3 $\times$.

weeks. Drying of the agar enhanced root formation and 73% (203/279) of the shoots from three experiments formed roots after 7 to 8 weeks. Survival of these rooted shoots in pots was 64% (36/56) in one trial, but 28% (26/42) in another trial because of a fungal infection.

This study demonstrated that in vitro shoot proliferation can easily be used to maintain germplasm of *C. macromeris* in culture and to rapidly produce many plants.

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