In Vitro Propagation of Mammillaria elongata

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Abstract. In vitro propagation of Mammillaria elongata DC plants was successful using tubercle explants grown on a medium based on Murashige and Skoog's high salts supplemented with various auxins and cytokinins. Optimum callus proliferation occurred in response to 2,4-dichlorophenoxyacetic acid (2,4-D) (2-10 mg/liter) with either kinetin or IAA (1 mg/liter). Root initiation was optimized with either naphthaleneacetic acid (NAA) or indolebutyric acid (IBA) (60 mg/liter). Shoots were transferred every 10 to 60 mg/liter and BA at 80 mg/liter. The auxin-to-kinetin balance required for shoot initiation appears to be unique for each species of Mammillaria studied. Shoots developed in vitro of M. elongata were successfully transferred to greenhouse conditions, where they rooted and continued to grow.

Since cacti normally are propagated by either seeds or cuttings, the application of tissue culture techniques to members of the Cactaceae would eliminate the required maintenance of large numbers of stock plants and would result in more uniform material eliminating seedling variability (2). Tissue culture propagation might also prove useful for maintaining populations of rare and endangered species (4), for reproducing cristate or monstrose forms, for reproducing the colorful shoots for grafting cacti, and for promoting breeding and hybridization through the quick commercial release of new types (2). This report describes the in vitro initiation and growth of callus, roots and shoots of Mammillaria elongata, and the successful establishment of these new plants in container culture under greenhouse conditions.

Previous investigations have shown that the basic regulatory mechanism for organogenesis in vitro is the balance of auxin to cytokinin (2, 7). Earlier attempts at in vitro culture of cacti utilized many different growth regulator additives (1, 3, 8). In this study 7 different growth regulators were used in various concentrations (0 to 100 mg/liter) to determine the minimum concentration(s) for producing the desired growth and organogenesis. Auxins tested were 2,4-D, NAA, IBA; cytokinins tested were 6-furfurylaminopurine (kinetin), and 6-benzylaminopurine (BA). These growth regulators were added to the basal medium before autoclaving. A minimum of 5 tube replicates were made of all concentrations of tested. The basal medium was Murashige and Skoog high salts (6) supplemented with Murashige, Serpa and Jones organic mixture (7) adjusted to pH 5.6. This medium is completely defined; earlier attempts with tissue culture of cacti used additives such as coconut milk (1, 3, 8). Twenty-five ml aliquots of the melted medium were dispensed into 25 x 150 mm culture tubes, stoppered with polypropylene tube closures, and autoclaved at 121°C at 6.8 kg/cm² pressure at 15 min. Explants were cultured for 8 weeks at 27° on a 16 hr photoperiod from cool white florescent bulbs at about 5 Rlx.

Many explants were tested (2), but the most reliable were tubercles with the spines trimmed (Fig 1). Tubercles are analogous to the leaf base of most angiosperms. Intact branches were removed from the stock plant and the spines were trimmed. The excised branches were soaked for 1 hr in a saturated benomyl solution with LOC³ detergent added, and were then surface sterilized for 30 min in a sodium hypochlorite solution (8 g/liter). The tubercles were then rinsed in sterile, distilled water before placing on the culture medium.

Callus proliferation (Fig 2) was stimulated by all auxins tested. Maximum callus proliferation occurred in response to 2-10 mg/liter 2,4-D with complementary levels of 1-2 mg/liter kinetin or 2iP. The callus was loose and friable, and proliferated quickly upon transfer. Moderate amounts of friable callus were also produced in response to complementary growth regulators, 1 mg/liter IAA and 10 mg/liter 2iP. Root initiation was promoted by both NAA and IBA with the maximum response to both growth regulators occurring at 60 mg/liter with complementary levels of 1-2 mg/liter kinetin or 2iP.

Shoot initiation was promoted to differing degrees by each cytokinins tested. Kinetin at 20 mg/liter, 2iP from 10 to 60 mg/liter and BA at 80 mg/liter yielded good responses. The most consistent shoot initiation occurred in response to the lower levels of 2iP, about 10.0 mg/liter, with a complementary auxin level of 1.0 mg/liter IBA (Fig 3). Shoots were transferred every 4 to 8 weeks and continued shoot growth as well as callus development at the base of the shoot (Fig 4). The callus produced at the base of the developing

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shoots was removed at each transfer and cultured for new shoot production or callus proliferation.

Once the shoots attained a size of 1 cm or larger, they were easily transferred to the greenhouse with 100% success after a 2 week conditioning procedure. For transfer, shoots without roots, were placed in small containers with soilless media, usually 2 peat: 1 vermiculite: 1 perlite and covered with a plastic bag. Small pin holes were made in the bag and these holes were enlarged over a 2 week period. During this period, the shoots adapted to greenhouse conditions and established a functional root system. Smaller shoots were also transferred, but the success rate was lower. This procedure resulted in almost uninterrupted growth of the new shoots (Fig 5).

One serious problem associated with tissue culture propagation of *M. elongata* is the high level of contamination. The cacti appear to host endogenous and/or exogenous pathogens which cannot be controlled by surface sterilization.

Tubercles of other *Mammillaria* species were placed into culture on 10 mg/liter 2iP and 1 mg/liter IBA which was optimum for shoot initiation. Results were variable, but good callus proliferation and root initiation were obtained from most cultures of one unidentified species. Both *M. spaerica* and *M. gracilis* responded with only small amounts of callus. *M. eichlamii* and another unidentified *M. sp.* showed no responses. These results suggest that each species of *Mammillaria* may require a specific and unique auxin: cytokinin ratio for specific responses which has been shown for other plant species (7).

**Fig. 1.** Tubercle explant of *Mammillaria elongata* with spines removed. **Fig. 2.** Callus proliferation in response to 2 mg/liter 2,4-D and 1 mg/liter kinetin. **Fig. 3.** Shoots initiation in response to 10 mg/liter 2iP and 1 mg/liter IBA. **Fig. 4.** Transferred shoot enlarging with additional basal callus prior to placement in the greenhouse. **Fig. 5.** Successful tissue culture plant of *M. elongata* growing under greenhouse conditions.

**Literature Cited**