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# In Vitro Propagation of *Paronychia chartacea*

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*Paronychia chartacea* Fernald (Caryophyllaceae) is an attractive cespitose, herbaceous plant with dichotomous branching. It grows in the central Florida scrub habitat and is listed as an endangered species by the federal government (Wood, 1990). Cultivated members of this genus are used in rock gardens, and they are easily propagated sexually and by division. In vitro techniques also have been used for the propagation of several Caryophyllaceae genera. Successful establishment of *Dianthus caryophyllus* L. (Frey and Janick, 1991; Kim and Kang, 1986), *Gypsophila paniculata* L. (Han et al., 1991; Kusey et al., 1980), and *Melandrium album* L. (Ye et al., 1992) have been reported; however, the wild *P. chartacea* population is steadily declining because of reduced seed production and viability primarily due to encroaching commercial development and agricultural land use (Christman and Judd, 1990). Therefore, our objective was to develop a micropropagation protocol to produce adequate plant material for eventual reintroduction studies in landscape preservation areas. In vitro propagation is becoming standard practice for endangered plant species where low or no germination is achieved using conventional techniques (Fay, 1992).

Nine seeds were collected from a single identified field specimen. They were surface-sterilized in 0.15 M NaOCl for 3 min and 12.2 M ethanol for 1 min. Seeds were rinsed three times with sterile deionized water and placed into individual 100 × 15-mm plastic petri plates containing 25 ml basal medium (BM). The BM consisted of Murashige and Skoog (1962) inorganic salts, 25 g sucrose/liter, and (in mg·liter$^{-1}$) 2 glycine, 0.5 nicotinic acid, and 0.1 pyridoxine hydrochloride plus 8 g Phytagar/liter (Gibco, Life Technologies, Grand Island, N.Y.). The medium was adjusted to pH 5.8 with 0.1 N KOH before being autoclaved for 20 min at 120C. The seed cultures were maintained at 23C under a 16-h, 90- to 98-µmol·s$^{-1}$·m$^{-2}$ photoperiod provided by cool-white fluorescent lamps. One seed germinated after 90 days, and the seedling was grown in vitro for 120 days. During this period, multiple shoot tips developed as a result of the dichotomous branching habit inherent to this species (Fig. 1). Shoot tips (3 mm long) were excised from the seedling and cultured on BM containing four concentrations (0, 2.2, 6.7, or 13.3 µm) of 6-benzylaminopurine (BA) for axillary shoot multiplication. The pH of each medium was adjusted to 5.8 before adding agar. Media were autoclaved for 15 min at 120C. Fourteen explants were cultured individually in 130 × 40-mm test tubes with each of the four BA concentrations in a completely randomized design. Cultures were maintained at 23C under a 16-h, 90- to 98-µmol·m$^{-2}$·s$^{-1}$ photoperiod provided by cool-white fluorescent lamps and evaluated for axillary shoot multiplication 45 days after culture initiation. Data were subjected to an analysis of variance.

Within 30 days of culture initiation, callus and multiple shoots developed from 0%, 29%, 21%, and 43% of the explants cultured with BA at 0, 2.2, 6.7, or 13.3 µM, respectively. BA concentration had a significant effect on the number of shoots formed per responding explant ($P \leq 0.01$; two, three, and five shoots for 2.2, 6.7, and 13.3 µM BA, respectively). The shoots were adventitious, originating from the callus. This organogenic response did not occur with explants cultured without BA; rather, these explants elongated with continual double-branching growth. This morphological characteristic of *P. chartacea* lends itself to a straightforward micropropagation system in the absence of growth regulator supplements. Thirty multiple side branches (1 cm long) subsequently were excised from the primary explants and transferred to BM without growth regulator supplements for root initiation and growth. Root development occurred on 87% of the shoots within 30 days (Fig. 2). The rooted plantlets were transferred to 52 × 26 × 6-cm flats containing potting soil that was autoclaved for 30 min at 120C. The flats were covered with a clear plastic dome and placed in a greenhouse under polypropylene shadecloth (50%) at a 300-µmol·s$^{-1}$·m$^{-2}$ average irradiance. The plants were kept in the covered flats for 40 days at 90% relative humidity (RH), measured by a PCRC-11 sensor (Phys-Chem Scientific, New York). Then, plants were transferred to 3.8-liter plastic pots and exposed to ambient RH (70%) and a 650-µmol·s$^{-1}$·m$^{-2}$ average irradiance. Of 24 plantlets transferred to the greenhouse, 23 survived.

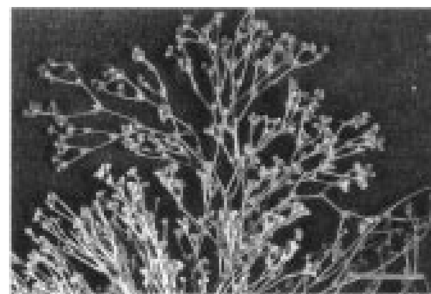

Fig. 1. Seedling of *Paronychia chartacea* exhibiting dichotomous branching habit. Scale bar = 5 mm.

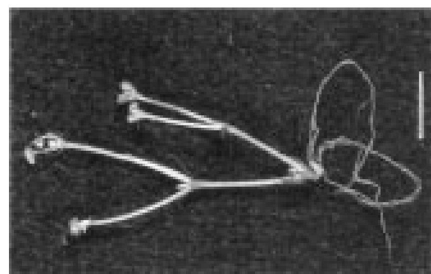

Fig. 2. Root development from an axillary shoot of *Paronychia chartacea* produced in culture and rooted in vitro. Scale bar = 10 mm.

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### Literature Cited

Christman, S.P. and W.S. Judd. 1990. Notes on plants endemic to Florida scrub. Fla. Scientist 53:52–73.

Fay, M.F. 1992. Conservation of rare and endangered plants using in vitro methods. In Vitro Cellular Dev. Biol. 28:1–4.

Frey, L. and J. Janick. 1991. Organogenesis in carnation. J. Amer. Soc. Hort. Sci. 116:1108–1112.

Han, B.H., K.Y. Paek, and J.K. Choi. 1991. Micropropagation of *Gypsophila paniculata* through shoot tip culture in vitro. J. Korean Soc. Hort. Sci. 32:394–400.

Kim, K.W. and M.S. Kang. 1986. Studies on the clonal multiplication of carnation through the in vitro cuttings. J. Korean Soc. Hort. Sci. 27:81–84.

Kusey, W.E., P.A. Hammer, and T.C. Weiler. 1980. In vitro propagation of *Gypsophila paniculata* L. 'Bristol Fairy'. HortScience 15:600–601.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473–497.

Wood, D.A. 1990. Official lists of endangered and potentially endangered fauna and flora in Florida. Florida Game and Fresh Water Fish Commission, Tallahassee.

Ye, D., Y. Wu, P. Installe, M. Jacobs, and I. Negrutiu. 1992. On the genetic control of the regeneration response in the dioecious *Melandrium album.* Sexual Plant Reproduction 5:292–297.