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Micropropagation of *Notholaena* 'Sun-Tuff Fern

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Abstract. **A micropropagation system was developed for micropropagation and rooting of *Notholaena* spp. Shoot cultures were initiated from mature sori on leaflets of the *Notholaena* cultivar Sun-Tuff and cultured on gelled Murashige and Skoog (MS) medium without hormones. Rooting and plant growth were evaluated on gelled MS, vermiculite moistened with liquid MS, or vermiculite moistened with water. Vermiculite wetted with MS was superior in promoting frond and root development. High humidity was not needed to acclimatize the plants ex vitro.**

'Sun-Tuff' fern is an attractive, slow-growing cultivar of *Notholaena* spp., collected in western Texas, that tolerates full sun and partially dry soils (Liberty Hyde Bailey Hortorium, 1976). The fern is propagated by division and ceases to grow for 1 month after division. Because of this physiological setback and its slow growth, production of large quantities of the fern is slow. Tissue culture propagation may alleviate the constraints of traditional propagation. In vitro propagation techniques have been applied to other ferns using runner tips, shoot apices, and isolated spores as explants (Beck and Caponetti, 1983; Cooke, 1977, 1979; Hicks and Von Aderkas, 1986; Knauss, 1976). The objective of this study was to develop a micropropagation system as an expeditious method for the multiplication of 'Sun-Tuff fern.

Leaflets containing mature sori were surface sterilized in 10% chlorine bleach for 15 min and rinsed three times with sterile distilled water to establish source cultures. Leaflets were cultured abaxial or adaxial side up on Murashige and Skoog (1962) medium (MS), containing 3% sucrose, 0.2% Gelrite (Merck and Co., Rahway, N.J.), at pH 5.8, dispensed as 50 ml/Magenta box (GA7 Magenta Corp., Chicago). Leaflets were cultured at 25 ± 2C under continuous 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light provided by cool-white fluorescent lights. After 6 weeks, the proliferating cultures from sori were transferred to fresh medium. Every 8 weeks thereafter, the clumps of plantlets were cut into fourths and subcultured.

Leaflets cultured adaxial or abaxial side down showed green spore development within 9 and 14 days, respectively. After 3 weeks, the proliferating gametophytes completely covered the adaxial side of the leaflets. The first fronds, distinguishable by petioles, developed after 6 weeks, and the first multileaflet fronds formed after 11 weeks in vitro. Cultures were comprised of dense masses of proliferating plantlets. As with several other fern species, no hormones were required for multiplication (Harper, 1976).

The in vitro rooting experiments with these plantlets were conducted in dram vials (9.4 × 2.2 cm). Treatments were 9 ml MS medium and 0.2% Gelrite, or MS medium and 1.46 g fine-grade vermiculite, or distilled water and 1.46 g vermiculite, all autoclaved for 20 min at 121C. Single plantlets were separated from 60-day-old cultures and planted one per vial. After 10 weeks, the plantlets were evaluated for size and number and length of fronds and roots. Each experiment had 15 replicates per treatment and was repeated once. Data were statistically analyzed to determine means and standard errors (Steel and Torrie, 1960).

Plantlets cultured on MS + vermiculite produced the largest plants, with numerous long roots (Table 1); those grown on gelled MS and water + vermiculite were similar, forming fewer fronds and few roots. MS + vermiculite also promotes optimal growth of cultured peach *[Prunus persica* (L.) Batsch] embryos, compared with gelled medium or filter paper support with liquid medium (Pinto et al., 1990). The vermiculite + MS probably promotes root growth because of better aeration than in a gelled medium. Increasing gelled medium poration, i.e., breaking it into chunks, stimulates in vitro root growth of *Trifolium subterraneum* cv. Mt. Barker (Barrett-Lennard and Dracup, 1988). In vermiculite wetted with aqueous MS, the sucrose could have provided supplemental carbohydrate, which would promote growth better than vermiculite wetted with water.

Plantlets grown in vitro in MS + vermiculite for 3 months were transferred to plastic boxes (22 × 22 cm) filled with 8 cm of either moistened vermiculite or a mixture of equal parts moistened peatmoss and perlite to determine survival following removal from culture. The boxes were covered with clear plastic lids to maintain high humidity and placed under 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of 18 h light, from cool-white fluorescent bulbs. After 2 weeks, the covers were removed and the plants were watered one or two times per week with distilled water for 8 weeks. Each experiment had 30 replicates and was repeated once. Percent survival and SE were calculated.

The survival rates of ex-vitro plants grown in covered boxes containing vermiculite or peatmoss-perlite were only 10% ± 1% and 20% ± 3%, respectively. Due to low survival rates in the high-humidity environment of the covered boxes, particularly with vermiculite, lower humidity was tested. Plantlets were transferred to plastic boxes filled with peatmoss-perlite and grown, without covers, under the conditions described above. Higher survival (80% ± 5%) was obtained with uncovered boxes. For most ferns, an initial period of high humidity is necessary to survive removal from culture (Cooke, 1979; Harper, 1976); but it was detrimental to this drought-tolerant fern.

'Sun-Tuff' can be successfully micropropagated by initiating plantlet development from sori on leaflets placed on gelled MS medium to induce plantlet development, then culturing plantlets on MS + vermiculite for optimal growth and root development, followed by planting in a peatmoss-perlite soil, without covering ex vitro.

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Table 1. Growth of 'Sun-Tuff' ferm cultured in Murashige and Skoog (MS) medium + 0.2% Gelrite, MS medium + 1.46 g vermiculite, or water + vermiculite, after 10 weeks.

	Plant measurements[z]				
Treatment	Plant survival (%)	Fronds (no.)	Frond ht[y] (mm)	Roots (no.)	Longest root (mm)
MS + 0.2% Gelrite	100 ± 0[x]	7.7 ± 0.3	15.7 ± 0.8	3.0 ± 0.5	3.0 ± 0.4
MS + vermiculite	100 ± 0	13.1 ± 1.0	21.6 ± 1.1	12.0 ± 0.8	15.7 ± 1.1
Water + vermiculite	80 ± 5	6.1 ± 0.3	8.6 ± 0.5	2.8 ± 0.2	4.2 ± 0.7

[z]Mean of three experiments, 15 replicates each, ± SE.
[y]Mean of three longest fronds from each plant.
[x]± SE.

Literature Cited

Barrett-Lennard, E.G. and M. Dracup. 1988. A porous agar medium for improving-the growth of plants under sterile conditions. Plant & Soil 108:294-298.

Beck, M.J. and J.D. Caponetti. 1983. The effects of kinetin and naphthaleneacetic acid on in vitro shoot multiplication and rooting of fishtail fern. Amer. J. Bot. 70:1-7.

Cooke, R.C. 1977. The use of an agar substitute in the initial growth of Boston ferns in vitro. HortScience 12:339.

Cooke, R.C. 1979. Homogenization as an aid in tissue culture propagation of *Platycerium* and *Davallia*. HortScience 14:21-22.

Harper, K. 1976. Asexual multiplication of leptosporangiate ferns through tissue culture. MS Thesis, Univ. of California, Riverside.

Hicks, G. and P. Von Aderkas. 1986. A tissue culture of the ostrich fern *Matteuccia struthiopteris* (L.) Todaro. Plant Cell Tissue Organ Cult. 5:199-204.

Knauss, J.F. 1976. A partial tissue culture method for pathogen-free propagation of selected ferns from spores. Proc. Fla. State Hort. Sci. 89:363-365.

Liberty Hyde Bailey Hortorium. 1976. Hortus third: A concise dictionary of plants cultivated in the United States and Canada. 3rd ed. Macmillan, New York.

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

Pinto, A.C.D., D.H. Byrne, and S.M.D. Rogers. 1990. Influence of in vitro conditions on growth of immature peach *[Prunus persica* (L.) Batsch] embryos. HortScience 25:1098.

Steel, R.G.D. and J.H. Torrie. 1960. Observations, p. 7-31. In: R.G.D. Steel and J.H. Torrie (eds.). Principles and procedures of statistics with special reference to the biological sciences. McGraw-Hill, New York.