Propagation of Ornamental Varieties of Spruce (*Picea* spp.) through Somatic Embryogenesis

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Somatic embryogenesis (SE) is a potent tool for rapid vegetative propagation and can produce high volumes of cloned embryos from a single seed. The impact of this new propagation technology on the ornamental conifer landscape industry may be substantial. Grafting of named cultivars is expensive and results in poor form requiring years of pruning and shaping. Somatic seedlings have the trueness-to-type of grafts, but also demonstrate the growth and form of true seedlings. We describe applications of SE for the commercial development of ornamental varieties of Colorado blue spruce (*Picea pungens*) and white spruce (*P. glauca*). At least 150 clonal lines of somatic seedlings have been established in nursery trials and cryopreservation. Trials will be assessed for landscape characteristics over the next several years prior to volume production of selected clones.

INTRODUCTION

Somatic embryogenesis (SE) is a relatively new technique for vegetative propagation of conifers and can produce high volumes of cloned embryos from a single seed. SE of conifers was first reported in 1986 (Hakman and vonArnold, 1985) and this tissue culture propagation technique has developed rapidly since then to the first pilot-scale forestry applications in recent years (Cervelli et al., 1994; Roberts et al., 1994). SE potentially offers a cost-effective replacement for other forms of vegetative propagation (Cervelli and Senaratna, 1994). At present, the technique is limited to the use of seed embryos or young seedlings as a source of tissue for induction of embryogenic culture lines. We describe applications of SE for the commercial development of ornamental cultivars of Colorado blue spruce (*Picea pungens*) and white spruce (*P. glauca*).

The impact of this new propagation technology on the ornamental conifer landscape industry may be substantial. Grafting of named cultivars is expensive and results in poor form requiring years of pruning and shaping. Somatic seedlings have the trueness-to-type of grafts, but also demonstrate the growth and form of true seedlings. In addition, clonal SE lines can be preserved indefinitely by storage in liquid nitrogen and bulked up for production at any time (Cyr et al., 1994). This unique ability to store clonal lines in this manner is significant because germplasm banks of superior material can be established and maintained at low cost while field trials are being performed. Production and delivery of somatic seedlings from the germplasm bank to the nursery can be accomplished within 6 to 8 months.

SE has been evaluated throughout the tissue culture, nursery production, and field establishment phases. Studies of the biochemistry and patterns of gene expression during development of somatic and zygotic embryos (Flinn et al., 1993) and during germination (Cyr et al., 1991) revealed that they are similar with respect to the accumulation and utilization of storage proteins and lipids. Assessment of genetic stability during SE using isozyme analysis (Eastman et al., 1991) and before and after cryopreservation using RFLP analysis (Cyr et al., 1994), indicate that somaclonal variation is very infrequent compared to other micropropagation methods (Scowcroft, 1984). Finally, intensive morphological and physiological assessments during nursery and field growth show that somatic seedlings are normal with respect to their performance and phenological patterns (Grossnickle et al., 1992; Grossnickle et al., 1994; Grossnickle and Major, 1994a and 1994b). At present we have established approximately 80,000 spruce somatic seedlings in nursery and field trials in at least six separate locations throughout North America, the oldest of which were field planted in 1990.

METHODS

Seed from the Kaibab and Piedra River provenances of Colorado blue spruce was obtained from Dean Swift Seed Co., Jaroso, CO. In addition, half-sib seed was obtained from the top four families ranked for color at the Michigan State University orchards established by the late Dr. Hanover. Full-sib white spruce seed of the variety Sunburst™ was obtained from the National Forestry Institute, Petawawa, Ontario. Sunburst™ spruce is characterized by a brilliant yellow spring foliage due to delayed chlorophyll development.

Seed were dissected and SE calli induced from seed embryos following the protocol described in Webb et al. (1989). These callus lines have been cryopreserved according to the protocol described in Cyr et al. (1994). Embryo maturation, desiccation, and germination were performed according to protocols described in Webster et al. (1990), with significant modifications (unpublished). Somatic seedlings were hand-potted to ray-leach tubes or by machine to 415B styroblocks containing a standard peat and perlite mix and grown in a controlled-environment room or greenhouse before transfer to the field. Styroblocks were planted by the Fast-Plant™ semi-automated planting technology developed for this purpose (Edmonds and Cervelli, 1993). The Fast-Plant™ machine utilizes a series of "open-books" of pre-moistened potting mix for the accurate and damage-free planting of 4- to 6-week-old somatic seedling germinants.

CURRENT STATUS AND DISCUSSION

SE is being applied to the establishment of germplasm banks of potentially valuable clones and nursery trials are being conducted to identify those clones with desirable landscape traits. Trials are currently being established as somatic seedlings are delivered from the laboratory. These seedlings are derived from clonal SE culture lines obtained from seed of both Colorado blue spruce and white spruce (Table 1). The induction frequencies of SE calli from dissected seed embryos ranged from 6% to 47%, depending on seed source and induction protocol (unpublished data). Cryopreservation of these lines have been achieved with greater than 80% success rate (unpublished data). In pilot production techniques, somatic seedlings are handled in a similar manner to true seedlings in the greenhouse and nursery.

Table 1. Current status (September 1994) of somatic embryogenesis (SE) culture and nursery trials of ornamental conifers.

	Colorado blue spruce			White spruce	
	Piedra River	Kiabob	Michigan State Univ.	Sunburst™	Total
# Seed dissected	1314	1601	932	1270	5117
# SE clonal lines in culture	116	57	57	87	317
# SE clonal lines in nursery trials	70	33	35	12	150

Both field and greenhouse trials of this material have been established in at least four separate locations representing different climates, soils, and cultural conditions. These locations include Nova Scotia, coastal British Columbia, western Maine, and western Michigan. These trials will be assessed for landscape characteristics, such as color and form, and winter hardiness over the next several years. Clones will be selected over this time for volume production.

Somatic embryogenesis offers a new potential for high-volume propagation of ornamental conifers. The technology provides genetic uniformity as with other forms of vegetative propagation, such as grafting or rooted cuttings, but the resulting propagule is identical in form and growth habit to a true seedling.

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QUESTION-ANSWER

Anonymous: What's the best way to do the irrigation?

Fred Rauch: In that particular case he waters them in very well and with the use of the plastic covering he's able to keep enough moisture in there especially for seeds that germinate quickly. If you had something extremely slow (several months) then this probably wouldn't be useful.

Anonymous: Do you have any strains from western Samoa?

Chuck Ades: No.

Anonymous: They have plants with leaves that are 15 to 18 in. long with beautiful variegations?

Chuck Ades: Do these characteristics hold in the United States?

Anonymous: They seem to.

Anonymous: What kind of insect problems to you have on your plants?

Chuck Ades: The main problem with pothos (*Epipremnum aureum*)is spider mites. Syringing the undersides of leaves gets rid of the mites.