DELIVERY OF GENETIC GAIN: CLONAL ESTABLISHMENT AND DELIVERY VIA SOMATIC EMBRYOGENESIS

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Somatic embryogenesis (SE) is a tissue culture method of propagation which can be used for multiplying top-ranked families or capturing a greater proportion of the additive, dominant or epistatic genetic gain through the selection of elite clones (Mullin and Park 1992). The traits which could be captured via SE are those which can be identified through any conventional breeding program. These include yield and wood quality, and resistance to stress, pest or disease. Additionally, SE facilitates the introduction of value-added traits through genetic transformation (Ellis et al. 1993).

In forestry, SE fulfills a role similar to rooted cuttings for multiplication of families. The advantages include an achievable lower production cost (Grossnickle et al. 1997) and storage of germplasm via cryopreservation (Cyr et al. 1994). The latter enables the regeneration of valuable genotypes for operational production following selection from clonal field trials. Embryogenic lines can be initiated from embryos of mature (spruce) or developing seed (Douglas-fir, pine spp.). Technology development has focused on various spruce species (Roberts et al. 1991, 1993; Park et al. 1993), lobolly pine (Handley et al. 1994), Douglas-fir (Gupta et al. 1994) and radiata pine (Smith et al. 1994). Since 1988, the Forest Biotechnology Centre (BCRI) and Silvagen Inc. have been active in the development of SE as a delivery system of elite genetic material for reforestation.

Interior spruce *(Picea glauca/engelmannii* complex) is a major commercial species in British Columbia (BC), Canada. A tree-improvement program implemented by the BC Ministry of Forests (BCMoF) has provided the opportunity to apply SE to the capture of height-gain (Sutton et al. 1993), and to take advantage of family and potential clonal differences in weevil resistance *(Pissodes strobi)* (Kiss and Yanchuk 1991, Alfaro 1996). The breeding, testing and selection of parents for additive gene effects is the main emphasis of the program (Kiss 1968). First-generation clonal seed orchards have been rogued and genetic gains were about 11% based on 10-year height results (Kiss and Yeh 1988). Clonal testing of progeny from selected parents will capture additional gain over and above gains from rogued seed orchards.

The Prince George (PG) Selection Unit, accounting for 60% of the 100 million Sx seedlings planted annually in BC, is the source of material for the interior spruce (Sx) SE clonal selection program. This was initiated in 1990 and utilized 12 full-sib families selected from a base population of 174 trees (Dr. G. Kiss, BCMoF). These families were ranked low, medium and high for growth and weevil resistance. Approximately, 250 embryogenic lines were produced and stored in cryopreservation at BCRI. Somatic seedlings (emblings) representing 181 lines were deployed in field trials during 1994-95 (K. Thomas, Dr. C. Hawkins, BCMoF).

A second clonal selection program for Sx was launched in 1993. This was devised to accelerate the selection of high-yielding and weevil-resistant genotypes by utilizing the top 5 to 10% of the 1st generation parents from the PG Selection Unit (Sutton et al. 1993). In brief, the design aimed at the field testing of 1,000 clones from 30 to 40 full-sib families on 2 to 3 sites selected on the basis of high-growth potential or high weevil-hazard. Based on a 5% selection intensity, a total of 30 to 50 clones will be chosen for operational implementation. The selection of value-added genotypes will be initiated at 5 to 6 years after initial outplanting. Since the previous clonal trial was based on the same 1st generation population, it is expected that genotypes from those trials will augment the operational population; the first selections will commence in 1999.

Approximately 1,900 Sx embryogenic lines representing 48 ranked families have been stored in cryopreservation since 1992. This clone bank has facilitated the delivery of 571 lines (21 full-sib families) to clonal field trials during 1996-97 (Dr. C. Hawkins, BCMoF). To increase genetic diversity, a new population of open-pollinated (OP) weevil-resistant families was added to the program (Quesnel Lakes). Fifteen top-ranked parents were selected from a base population of 140 trees based on progeny field tests for weevil resistance (Dr. R. Alfaro, Natural Resources Canada [NRCan] and Dr. G. Kiss, BCMoF). Based on current nursery inventories, an installment of up to 700 lines from 15 families for the 1988 clonal field trials is expected. This will ensure a delivery of up to 1,200 lines from 48 families for the 1996-98 clonal selection program. Additionally, a subset of the families and lines represented in the 1996 trials were installed in weevil screening trials by Dr. Rene Alfaro (NRCan).

Sitka spruce (Ss: *Picea sitchensis)* is a valuable coastal species which has been decimated by the pine weevil. The annual planting has declined by more than 90% from a historical high of 10 million seedlings per annum. Consequently, less-desirable species such as western hemlock and western red cedar are being planted as replacements. In response to these pressures on coastal reforestation, a breeding program for weevil-resistant Sitka spruce has been initiated during the past decade (King 1994).

A SE program for weevil-resistant Ss started at BCRI in 1994, has resulted in a clone bank of 322 lines representing 22 weevil-resistant families, primarily open-pollinated. Emphasis is being placed on the highest ranked of 75 tested OP families (Jordan River Field Trial, Dr. J. King, BCMoF; Dr. R. Alfaro, NRCan). Clonal trials of 42 lines (11 families) were initiated in 1997 in collaboration with, the BCMoF (C. Cartwright, Dr. J. King), forest industry partners (Canadian Forest Products, International Forest Products, Macmillan-Bloedel, Western Forest Products, Weyerhaueser) and the Oregon Department of Forestry. Additionally, collaborations have been established for weevil-screening (Dr. R. Alfaro, NRCan) and cuttings protocol development (D. Summers, BCMoF). This Ss program is expected to accelerate, with approximately 175 lines targeted for deployment in 1998 clonal trials. The induction of new embryogenic lines from the top-ranked families is in progress (1999 clonal trials). This will facilitate increasing the clone bank to 500 lines.

Efforts at BCRI are now being focussed on SE programs for other conifer species. For Douglas-fir *(Pseudotsuga menziesii),* 250 embryogenic lines from 10 high-yield families have been stored in cryopreservation. This complement is expected to increase by 100 lines during

the summer of 1997. Additionally, embryogenic lines have been established for several pine species with 170 of 400 of the lines currently stored in the clone bank.

Sx and Ss have been scaled to commercial production by Silvagen Inc. during the past 18 months with over 750,000 SE propagules produced to date. In 1997, 200,000 Sx somatic seedlings (spring and summer-ship) are being delivered to operational trials, while 180,000 Sx (12 full-sib families) and 15,000 Ss (12 lines) currently in nursery production for deployment in 1998. Pilot-scale production has demonstrated a current capacity of over 1 million somatic embryos annually. Deployment guidelines (under development by the BCMoF), currently require an effective population of 10 and 5 lines per full-sib family; the SE production for 1998 stocks will satisfy these requirements.

Keywords: *Picea glauca engelmannii, Picea sitchensis, Pseudotsuga menziesii,* pine, somatic embryogenesis, selection, clone bank, genetic gain.

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