The Impact of Variable Success of Somatic Embryogenesis Among Elite Crosses on Expected Genetic Gain and Diversity of Selected Varieties

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From its early beginnings in the 80's, somatic embryogenesis of conifers has progressed to commercial status in some species (Cyr and Klimaszewska 2002). The current challenge is to produce large numbers of lines for screening and verification of genetic value. Crosses between elite parents selected from advanced breeding programs are used to produce embryogenic cultures in the laboratory, from which somatic seedlings can be propagated. Viable plants recovered from these cultures are planted in varietal tests. The goal is to select and multiply outstanding varietal lines, and to plant these operationally in forest plantations.

Although this technology offers potential for larger genetic gains compared to other deployment schemes, only a fraction of genotypes entering the laboratory culture phase (induction) actually result in viable somatic lines. Individual crosses often vary widely in their ability to generate embryogenic cultures under any given laboratory protocol (MacKay et al. 2001; Pullman and Johnson 2002). The objective of this study was to quantitatively evaluate such differences in embryogenic success, in terms of expected genetic gain in selected varietal mixtures.

METHODS

Stochastic simulation of a recurrent selection program included the generation of elite crosses, followed by testing and selection of superior varietal mixtures (Figure 1).

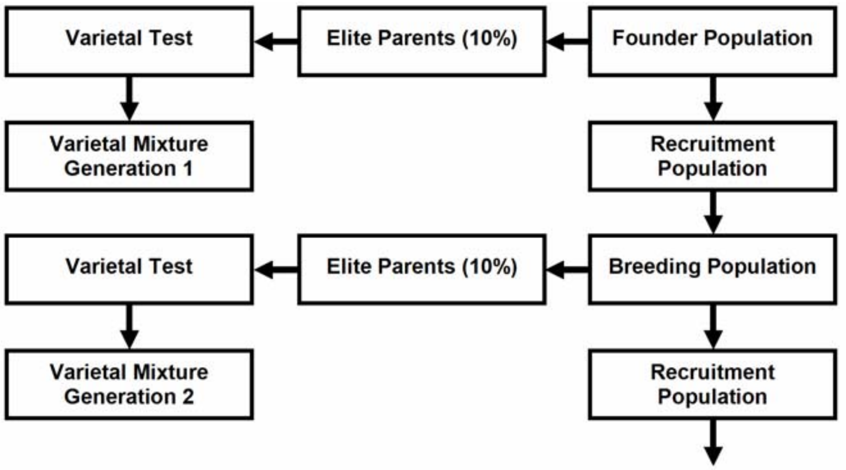

Figure 1. Schematic description of the recurrent strategy considered in this study.

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The recurrent program was initiated by generating 100 founder genotypes "founder population". Phenotypic observations were due to independent additive genetic effects and environmental deviations. The initial value of narrow-sense heritability was set at 0.3. Single-pair mating was performed among founders (50 controlled crosses). 50 progeny genotypes were generated in each full-sib family "recruitment population" and planted in a test, where each progeny genotype was replicated by ten ramets (10 independent environmental deviations). Following the test evaluation, top two individuals per family were selected to serve as parents in the following generation "breeding population". This process was repeated for 6 generations. Simulation was done using POPSIM^{TM} software, which is based on infinitesimal model assumptions (Mullin and Park 1995).

In every generation, top 10% of parents were selected (denoted as "elite parents") and crossed in a half-diallel mating. Progenies of these crosses entered the embryogenic induction and testing phase of the program. Success in somatic embryogenesis among crosses was considered either constant (**CONST**) or varied as an exponential distribution of family sizes. In the latter case, success rate was distributed among crosses either at random (**EXP-RAND**) or as a function of expected family values (**EXP-BV**). Regardless of the distribution of induction success rate among the elite crosses, the total number of lines field tested was fixed (2,000). The top ten lines were selected and the expected gain of this mixture reported.

RESULTS AND DISCUSSION

Surprisingly, variation in embryogenic propensity (i.e., induction success rates) among elite crosses had a negligible impact on the average genetic gain from the selected varieties (Figure 2).

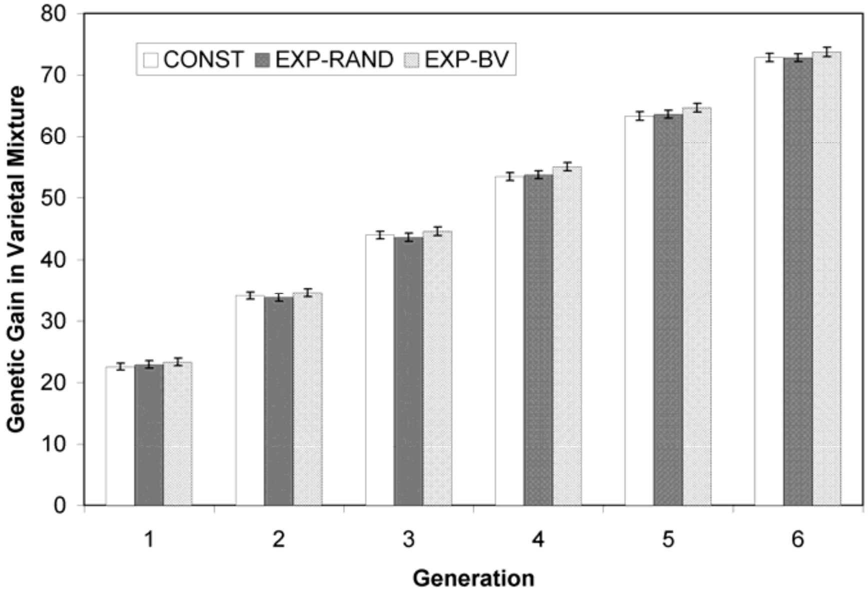

Figure 2. Genetic gain in individual generations (1-6) for the three scenarios tested in this study. Bars represent average values of 400 simulation iterations along with confidence intervals (alpha = 0.05).

This observation holds across generation due to the relatively small amount of genetic variation <u>among</u> elite families, as opposed to large <u>within</u>-family variation, such that there is a large potential to select outstanding varieties, even though they may have not have originated from crosses among the very best of the elite parents. The additive variance within families is replenished in every generation and is a function of the initial additive variance (in unselected population) and the inbreeding of parents (which was also considered in this study). The expected gain in selected varietal mixtures

In reality, varietal tests in individual generations will be reevaluated over time as the tests mature. This could lead to revised selections of varieties used in large-scale plantations. At a certain time, new test will become available (from crosses among elite parents in next generation) and the selection will concentrate on these.

It can be expected that as the technology of somatic embryogenesis further develops, this will lead to the normalization of responses among elite lines (Klimaszewska and Cyr 2002). The results of this study indicate that following such normalization and redirecting resources according to expected family values (scenario EXP-BV) would not necessarily lead to an important increase in genetic gains, if the testing effort (overall propagation success) is fixed.

The results from the present study support directing the breeding efforts of cloning programs towards conducting larger number of crosses rather than the common practice of concentrating on the top parents. The use of more crosses increases the chance for capturing superior genotypes through within families' selection as well as maintaining wider genetic diversity, an important consideration for clonal forestry deployment programs. The observed lack of substantial genetic differences among families when fewer crosses are made reduces the potential gain from these programs. Additionally, the observed minimal impact of family size (i.e., number variety produced/cross) among the implemented three scenarios (**CONST, EXP-RAND** and **EXP-BV**) on the captured gain demonstrates that the energy invested on maximizing the induction rate requires further evaluation.

REFERENCES

Cyr, D.R., and Klimaszewska, K. 2002. Conifer somatic embryogenesis: II. Applications. Dendrobiology 48: 41-49.

MacKay, J., Becwar, M., Park, Y.-S., Perfetti, C., Cordero, J., Pullman, G., Lockart La'C. 2001. Genetics of somatic embryogenesis in Loblolly pine. *In* Proceedings of the 26th Southern Forest Tree Improvement Conference, 26-29 June 2001, Athens, Georgia, USA. *Edited by* J.F.D. Dean. Pub. 48, Southern Forest Tree Improvement Committee. pp. 40-47.

Klimaszewska, K., and Cyr, D.R. 2002. Conifer somatic embryogenesis: I. Development. Dendrobiology 48: 31-39.

Mullin, T.J., and Park, Y.S. 1995. Stochastic simulation of population management strategies for tree breeding: a new decision-support tool for personal computers. Silvae Genet. 44: 132-141.

Pullman, G.S., and Johnson, S. 2002. Somatic embryogenesis in loblolly pine (*Pinus taeda* L.): improving culture initiation rates. Ann. For. Sci. 59: 663-668.