The impact of differential success of somatic embryogenesis on the outcome of clonal forestry programs. I. Initial comparison under multitrait selection

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Abstract: A breeding program where clones or clone mixtures are deployed through somatic embryogenesis of superior genotypes produced from elite-parent crosses was evaluated by simulation. A recurrent-selection scheme for general combining ability was considered with a breeding population size of 100 individuals. The population was assortatively pair-crossed with offspring cloned in a progeny test to facilitate forward selection of the next generation. Apart from crosses for the population advancement, "elite" crosses were made among 10 top-ranking individuals in each generation. These crosses differed in their propensity to produce somatic embryos (defined as induction success rate); the impact of this variable propensity on genetic response for two traits in selected 10-clone deployment mixtures was evaluated. The two traits considered in this study can be regarded as "productivity" and "quality". The results revealed that variation in success of clonal propagation does not necessarily lead to a reduction in potential genetic gains from selected clonal mixtures. This can be explained by the relatively small variation that exists among elite crosses, as opposed to large within-family variation. This large within-family variation provides ample potential for selecting superior offspring genotypes, even though they may have not originated from crosses among the very best of the elite parents. This conclusion holds for a range of heritability, correlations between traits, and their relative economic importance.

Résumé : Les auteurs ont évalué, à l'aide de simulations, un programme d'amélioration génétique où le déploiement des clones ou des variétés multiclonales se faisait par l'intermédiaire de l'embryogenèse somatique de génotypes supérieurs issus de croisements entre parents d'élite. Une stratégie de sélection récurrente pour l'aptitude générale en croisement a été considérée à partir d'une population d'élevage constituée de 100 individus. La simulation impliquait des croisements biparentaux spécifiques, la multiplication végétative des descendants et leur établissement dans un test de descendances afin de permettre la sélection par en avant pour le prochain cycle d'amélioration. En plus des croisements précédents, des croisements « d'élite » ont été simulés parmi les 10 meilleurs individus à chaque cycle. Ces croisements étaient différents quant à leur propension à produire des embryons somatiques (définie comme le taux de succès en induction). L'impact de cette propension variable sur la réponse génétique de deux caractères utilisés pour effectuer la sélection en vue du déploiement de variétés formées de 10 clones a été évalué. Les deux caractères considérés dans cette étude étaient représentatifs de la « productivité » et de la « qualité ». Les résultats ont montré que la variabilité dans le succès obtenu avec la multiplication végétative des clones ne s'est pas nécessairement traduite par une baisse de gains génétiques potentiels chez les variétés multiclonales sélectionnées. Ce résultat peut être expliqué par la variabilité relativement faible qui existe entre les croisements d'élite, comparativement à l'importante variabilité remarquée à l'intérieur des familles. Cette variabilité intrafamiliale permet une grande marge de manœuvre pour sélectionner des génotypes de descendants supérieurs, même s'ils ne proviennent pas nécessairement de croisements entre les meilleurs parents d'élite. Cette conclusion est valide pour une gamme de valeurs d'héritabilité, de corrélations entre les différents caractères considérés et leur poids économique relatif.

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Introduction

Since the first published reports on conifer somatic embryogenesis (SE) in the 1980s (Chalupa 1985; Hakman et al. 1985; Nagmani and Bonga 1985), SE has been accomplished in many species and hybrids, primarily of the family Pinaceae. Work with some species has already evolved into large-scale clonal selection programs (for review, see Cyr and Klimaszewska (2002), pp. 42–43). Such programs focus on elite genotypes developed in breeding programs with the goal to obtain embryogenic cultures of their offspring genotypes and recover from these cultures viable plants that are planted into clonal tests, followed by mass propagation and deployment of superior tested clones.

The implementation of SE may realize larger gains compared to seed-orchard technologies because of higher selection intensity and capture of nonadditive genetic effects. The propagation effort can focus on a few elite parents, and additional improvement can be achieved by practicing within-family selection in test families (Sutton 2002, Fig. 3). The testing stage can take considerable time, during which the embryogenic cultures must be maintained in cryostorage (Park 2002). However, factors such as genetic variation in cultural responses, developmental stage of embryos, embryo handling and storage, and culture medium may all result in losses of embryo yield. As a result, only a fraction of the genotypes entering the laboratory-culture phase of the program may result in successful embryogenic lines, and propagation success may not correlate with the commercial value of these lines (Park et al. 1993; Timmis 1998; Klimaszewska and Cyr 2002; Niskanen et al. 2004). Variation in success among families is frequently observed in the laboratory and is a major obstacle to generation of viable SE lines from many elite crosses. This variation in culture initiation was investigated in a diverse set of loblolly pine (*Pinus taeda* L.) families and was found to be under genetic control (MacKay et al. 2001).

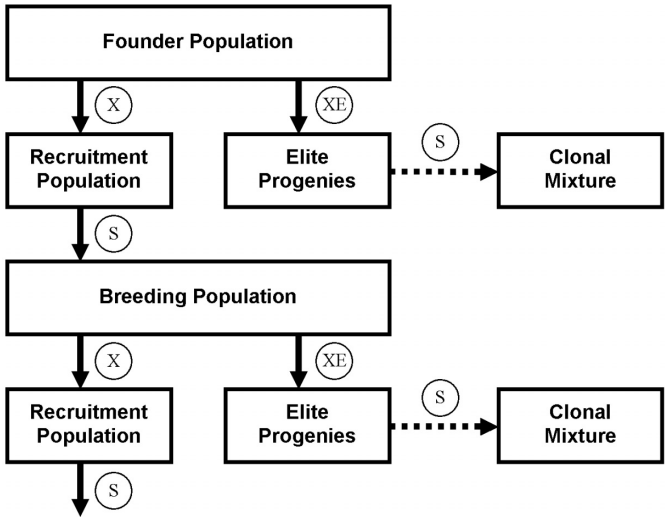
The objective of this study was to evaluate the influence of the variation in success of SE among individual crosses on the outcome of a clonal selection program, where clones are deployed to plantations by SE. In this study, we focus on the variation in the induction success rate, which is an expression of the number of clones (cell lines) captured from a particular elite cross (full-sib family). In forest tree breeding, the improvement of multiple characters is usually of interest (e.g., Danell 1995; McKeand and Bridgwater 1998; Mikola 2002). Two traits (productivity and quality) are therefore considered and the impact of their covariance and economic importance on the study's objective is evaluated.

In a follow-up study (part II), additional sets of assumptions will be investigated with respect to the objectives exemplified in the current paper. Specifically, we are interested in evaluating whether the present study's conclusions hold under lower test resources (i.e., lower number of induced lines from elite crosses) and variable numbers of selected clones in the variety mixture.

Methods

A schematic description of the simulated breeding strategy is provided in Fig. 1. The forest tree breeding simulation tool POPSIM™ (Mullin and Park 1995) was used and modified as described next. The phenotypic value of each tree was composed of an independent additive genetic component and an environmental deviation. Two conceptual traits were considered throughout this study: productivity and quality.

Fig. 1. Schematic description of the conceptual breeding strategy implemented in the present study. The first two generations are illustrated; subsequent generations follow an identical plan. Symbol "X" designates single-pair mating among all breeding individuals (100 individuals, 50 full-sib crosses). In addition to these crosses, the 10 top-ranking breeding individuals also contributed to generating the clonal mixture for mass deployment. These additional "elite" crosses are designated "XE" and were performed using half-diallel mating, with 45 crosses in total. Symbol "S" refers to selection. The dotted arrow designates that some time delay is expected between the assessment of elite progenies and the mass production of viable clonal plants for the deployment.



Simulating multiple traits

When multiple traits are considered in a parametric simulation model, a set of random variates is drawn from a multivariate normal distribution. The expected variances and covariance of these variates were supplied as an input in matrix $\mathbf{V}_I$. A triangular matrix $\mathbf{R}$ was then generated by the Cholesky decomposition such that $\mathbf{RR}' = \mathbf{V}_I$ (Gentle 1998). The lower triangular elements of matrix $\mathbf{R}$ for the general case of two variates can be expressed as

$$[1] \qquad \mathbf{R} = \begin{bmatrix} \sigma_1 & \\ \sigma_2\sigma_{1,2} & \sigma_2\sqrt{1-\sigma_{1,2}^2} \end{bmatrix}$$

where σ_1 and σ_2 are designated as the standard deviations of variates one and two, respectively, and $\sigma_{1,2}$ is the designated covariance between the two variates. Since the task was to generate variates with expected variances equal to one, the triangular matrix $\mathbf{R}$ is further simplified into

$$[2] \qquad \mathbf{R} = \begin{bmatrix} 1 & \\ \sigma_{1,2} & \sqrt{1-\sigma_{1,2}^2} \end{bmatrix}$$

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The generation of input random samples consisted of generating independent random numbers from the uniform distribution. The random number generator implemented in this study was MRG32k3a (L'Ecuyer et al. 2002). These random numbers were then transformed as described in Press et al. (1992, p. 280) to normal variates u: $u \sim N(0, 1)$.

Additive effects of founder genotypes ($\mathbf{A}_F$) were then sampled from the multivariate normal distribution, such that

$$[3] \qquad \mathbf{A}_F \sim N(0, \mathbf{V}_F);\ \mathbf{V}_F = \begin{bmatrix} \sigma^2_{A1} & \sigma_{1,2} \\ \sigma_{1,2} & \sigma^2_{A2} \end{bmatrix}$$

where σ^2_{A1} and σ^2_{A2} are initial additive variances for traits one and two, respectively. To generate the additive effect of each trait n in each ith founder genotype, the following expression was used

$$[4] \qquad \mathbf{A}_{Fni} = \sum_{j=1}^{n\ \text{trait}} \mathbf{R}_{nj}\, u_{ji}\, \sigma_{An}$$

Additive effects of offspring genotypes ($\mathbf{A}_P$) generated in each breeding generation were sampled from the multivariate normal distribution, such that

$$[5] \qquad \mathbf{A}_P \sim N(\boldsymbol{MPA}, \mathbf{V}_o);\ \mathbf{V}_o = \begin{bmatrix} 0.25(2 - F_f - F_m)\,\sigma^2_{A1} & \sigma_{1,2} \\ \sigma_{1,2} & 0.25(2 - F_f - F_m)\,\sigma^2_{A2} \end{bmatrix}$$

where ***MPA*** are mid-parent additive values for full-sib families corresponding to trait n. The matrix $\mathbf{V}_o$ is due to the Mendelian sampling of genes (giving rise to within-family genetic variation), where F_f and F_m are inbreeding coefficients for female and male parents, respectively. The additive effect of each trait n in each ith offspring genotype was generated (modified from del-Bosque González 1989) as

$$[6] \qquad A_{Pni} = \frac{A_{fn} + A_{mn}}{2} + \sum_{j=1}^{n\ \text{trait}} \mathbf{R}_{nj}\, u_{ji} \sqrt{\frac{1 - 0.5(F_f + F_m)}{2}\,\sigma^2_{An}}$$

Each offspring genotype was clonally replicated by 10 ramets. The environmental effect of each ramet for each trait n was randomly sampled from $N(100, \sigma^2_{En})$, and thus the environmental deviation did not contribute to the correlation between traits. The trait means were arbitrarily set to 100; other values could have been used with no effect on results. The initial variances (σ^2_{An}, σ^2_{En}) were supplied as a simulation input parameters, such that the phenotypic variance for each trait was equal to 500, and the narrow-sense heritability for the two traits was set to $h^2_1 = 0.1$ and $h^2_2 = 0.2$, or $h^2_1 = 0.3$ and $h^2_2 = 0.6$, for productivity (trait 1) and quality (trait 2), respectively. The correlation between traits was varied over the range –1 to +1 in individual simulation scenarios.

Hypothetical breeding strategy

Breeding was initialized by selecting 100 founder genotypes (founder population) from a population of unrelated and noninbred individuals (natural stands). In the case of outcrossing forest tree species, it seems appropriate to assume that the initial breeding population is composed of unrelated and noninbred individuals. Assuming that no selection was applied to the sampling of the founder population simplifies the simulation by avoiding an additional input variable (point of truncation). A two-trait index value was calculated for each tree in the founder population. In this calculation, it was assumed that the true genetic variances were known and that the additive correlation among traits was not affected by continuous selection. In reality, only estimates of these parameters are available, but the index seems to be generally robust in this respect, given that a considerable amount of data is available for estimation (Harris 1964). Further, it is not an objective of this study to assess the efficiency of genetic evaluation; it was expected that estimates fit closely to the true parameters. Let $\mathbf{P}$ be the variance–covariance matrix of phenotypic records, $\mathbf{C}$ the matrix of covariances between the sources of information in the selection index and additive genetic values in the aggregate breeding value, and $\boldsymbol{a}$ the vector of economic values for both traits. The vector of index weights $\boldsymbol{b}$ (partial regression coefficients) was then calculated as

$$[7] \qquad \boldsymbol{b} = \mathbf{P}^{-1}\mathbf{C}\boldsymbol{a}$$

Trees in the founder population were sorted by this index value and positive-assortative single-pair mating was performed to generate 50 controlled crosses (between trees with ranks 1 and 2, 3 and 4, etc.). For each of these crosses, 50 offspring genotypes were generated (as described previously), each clonally replicated by 10 ramets. These were planted in a field test and are referred to as the "recruitment population", as they are later used as selection candidates.

Following the assessment of all clonal offspring (selection candidates) and the calculation of selection index values, the two top-ranking (based on their two-trait index value) offspring clones were selected from each family (balanced within-family selection) to form the next generation's breeding population (BP). The breeding–testing–selection process as described previously was repeated over six generations. The sorting of mates prior to mating was based on the index value only; no restriction on relatedness was imposed in this respect.

Production population (PP)

The clonal deployment implemented in this study was basically identical to that illustrated by Park (2002, Fig. 1). Independent from the clonally replicated progeny tests generated as a basis for the population advancement (recruitment population), additional "elite" crosses were performed in every generation among the 10 top-ranking trees in the BP using a half-diallel mating design with 45 crosses in total (selfing was excluded). The purpose of these elite-parent crosses was to generate candidate clones for mass deployment to clonal forest plantations. The distribution of the induction success rate (family sizes) for these elite crosses was derived as follows. First, five families were randomly selected and assigned family size equal to zero (representing families whose induction rate in the laboratory was a complete failure). Second, 40 random values were sampled from the uniform distribution using the MRG32k3a random number generator (L'Ecuyer et al. 2002) and transformed to obtain exponentially distributed values $t \sim \text{Exponential}(\lambda)$, (Casella and Berger 2002, pp. 247). These exponential values were then randomly assigned to individual families. The

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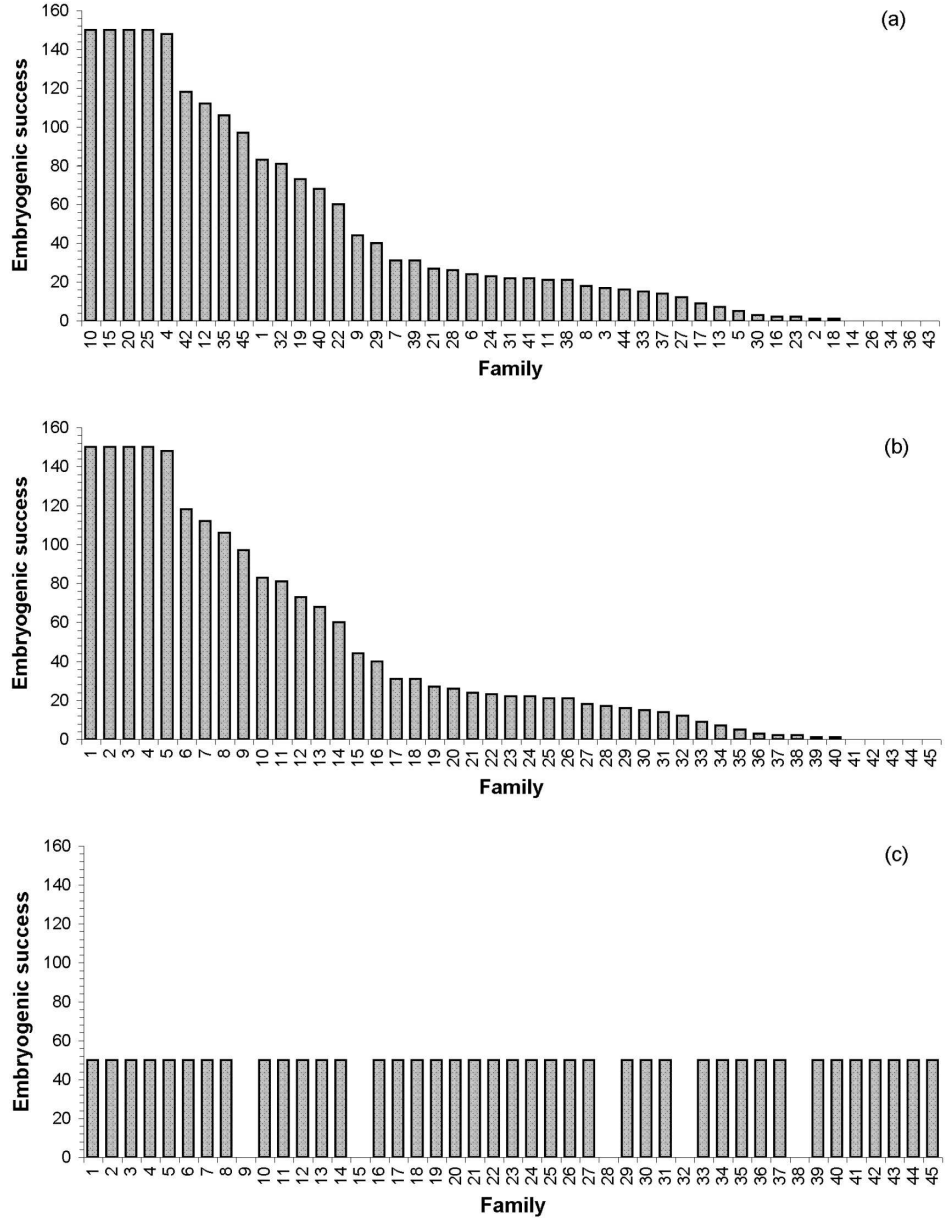
Fig. 2. Example of somatic embryogenesis induction success among elite crosses (full-sib family sizes). The success was either derived from the truncated exponential distribution or assumed to be constant (Fig. 2*c*, alternative C). The rank number of each full-sib family is provided on the *x*-axis. Under truncated exponential distribution, families were assigned success rates in proportion to expected family values (Fig. 2*b*, alternative E(BV)) or, alternatively, at random (Fig. 2*a*, alternative E(RM)). Parameter λ was iterated so that the total number of cell lines for clonal testing reached 2000, while satisfying the restriction of maximum family size equal to 150. Different values were obtained with different sequences of random numbers in individual generations and iterations of simulation scenarios.



maximum size of each family was limited to 150 cell lines (induced genotypes); sizes exceeding this maximum were truncated. Considering this restriction, parameter λ was iterated, such that the overall number of induced lines from all crosses reached 2000, averaging 50 genotypes per cross (full-sib family). An example of such a distribution of family sizes is provided in Fig. 2. Each offspring genotype was clonally replicated for clonal testing by 10 copies (ramets).

The random assignment of exponential values to individual families just described refers to the set of scenarios of the first alternative ("E(RM)"). This alternative simulates the random variation in SE success among families that might be expected in a laboratory setting applying a generalized propagation protocol. Two additional sets of scenarios were conducted for comparison. In the first set, the generation of family sizes was as described previously; however, exponential random variates were not assigned to families at random, but rather followed the ranking based on mid-parent index value, that is, predicted breeding values (alternative 2, "E(BV)"). This alternative simulates the situation where SE success is truly correlated with breeding value or where the laboratory has deliberately sought to achieve greater numbers of SE lines from those families with higher expected performance. Finally, a third set of scenarios was run with

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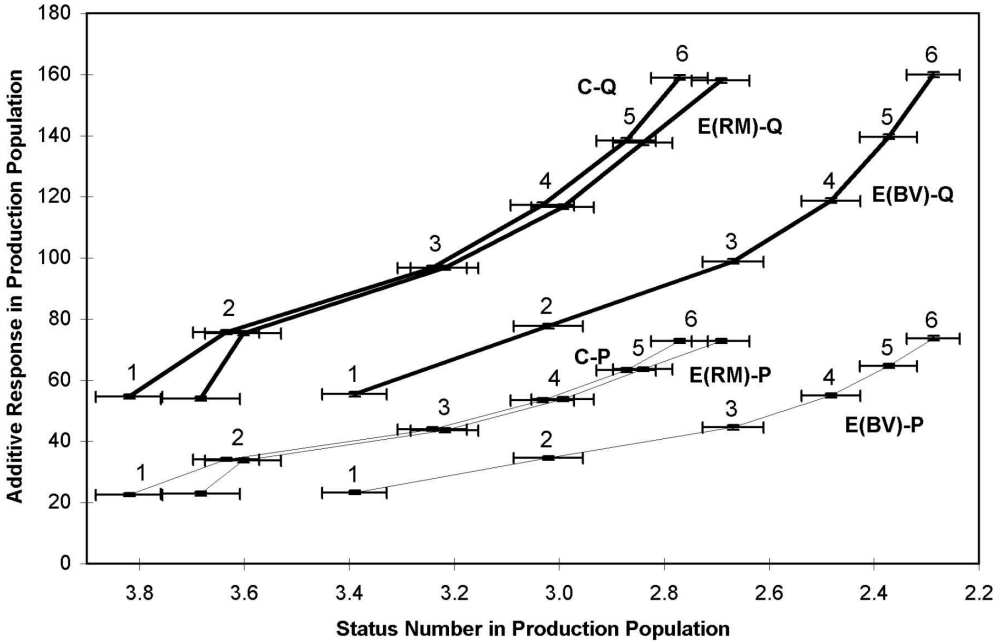
Fig. 3. Additive genetic response and effective population size (status number) in the production population in individual generations (labeled one through six). Three distributions of embryogenic induction success are compared in the graph: constant (C); exponential with random allocation of induction success to elite families (E(RM)); and exponential with induction success proportional to the expected family value (E(BV)). Symbols P and Q refer to the productivity (thin line) and quality (thick line) traits, respectively. Lines connect average values of 400 simulation iterations of a given scenario, plotted with 95% confidence intervals. The comparison corresponds to the case where the heritability for productivity $h^2(P) = 0.3$ and that for quality $h^2(Q) = 0.6$. Correlation between traits (r) was 0.1, and economic weights on both traits were equal ($a_1 = a_2 = 0.5$).



equal numbers of SE lines from each family (exactly 50 genotypes in each of 40 families, alternative 3, "C"). For all alternatives, the deployment mixture was composed of the 10 highest-ranking clones, based on two-trait index values; relatedness was not considered when selecting these clones.

Simulation and evaluation of results

The simulation was replicated by 400 independent iterations. Averages of genetic effects, corresponding variances, inbreeding coefficients and effective population sizes in each generation were calculated across all iterations. Averages of genetic effects and inbreeding coefficients were plotted as a function of effective population size, and the three alternatives described previously were compared. The effective population size used for the evaluation of results was measured by "status number" (N_S), which is the census size of an equivalent population composed of unrelated, noninbred individuals, where the probability to draw two genes that are identical by descent is the same as for the population under study. Status number was derived as half the inverse of group coancestry, which was calculated as the average of all pair-wise and self-coancestries of respective individuals (Lindgren et al. 1996).

Results

Throughout all scenarios, the additive genetic response from the selected clonal mixture was only marginally affected by the alternative distributions of embryogenic induction success rate (Fig. 3 illustrates this point). The difference between the exponentially distributed induction success with random allocation of family sizes (alternative E(RM)) and constant induction success (alternative C) in genetic response was not statistically significant ($p > 0.05$). While there appeared to be a tendency for higher values of N_S under C, under the majority of cases these differences were also not significant. In addition, there was considerable run-to-run variability in N_S (assessed by confidence intervals), so that for any two iterations of the same scenario the relationship between E(RM) and C with respect to N_S is essentially not predictable. The observed similarity between E and C distributions in individual-generation outcomes was independent of other input parameters in this study that are described separately next. When the exponential allocation of induction success followed the ranking based on expected family values (alternative E(BV)), there was an increase of approximately 1% in additive genetic response, accompanied by a significant decrease in N_S (N_S in generation 6 was 2.7 under E(RM), but 2.3 under E(BV)) (Fig. 3).

The effect of narrow-sense heritabilities of both traits in the BP and PP is illustrated in Fig. 4. Higher h^2 provided stronger genetic responses for both traits in the BP at the same N_S (balanced within-family selection). In the PP, higher h^2 led to greater gains as well as lower N_S, although this reduction in N_S was statistically significant only in later generations (5 and 6).

After an initial decline, the additive genetic variance $V_{A(BP)}$ increased over generations in the BP due to the positive assortative mating. The $V_{A(BP)}$ values for the quality trait in generation 6 were 420.8 and 144.0 at $h^2 = 0.6$ and 0.2, re-

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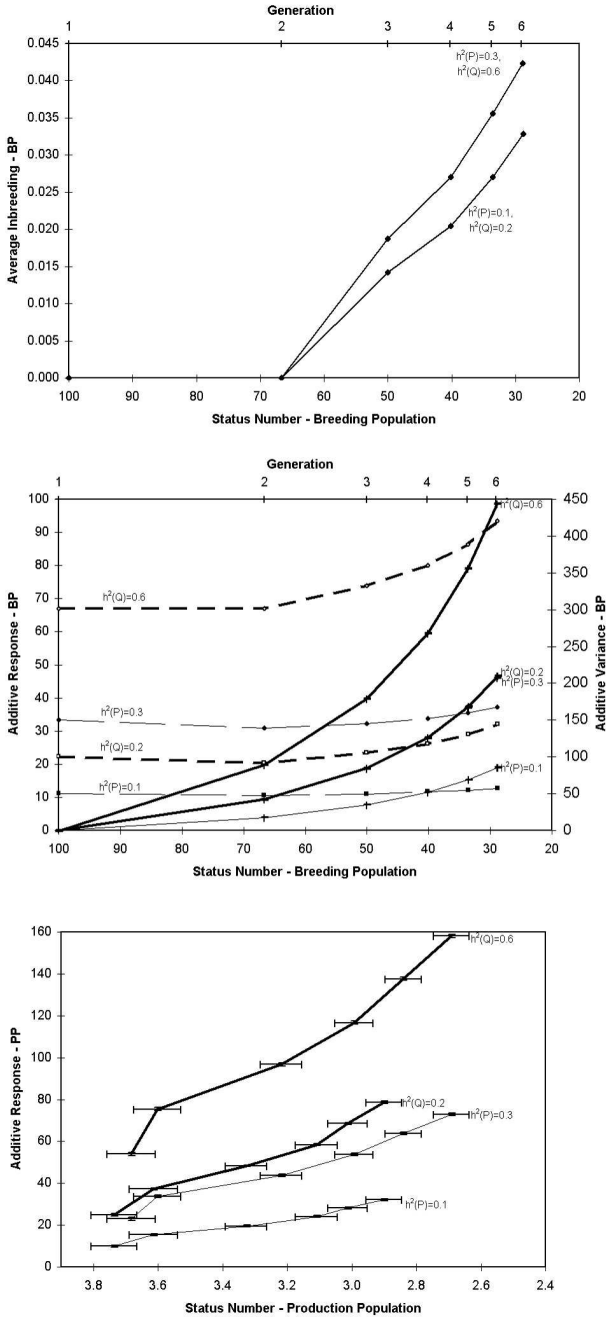
Fig. 4. Additive genetic response (solid line), additive variance (broken line), average inbreeding coefficient, and effective population size (status number, *x*-axis) in the breeding population and average genetic response in the production population (lower graph) in individual generations, for the respective traits. Different values of heritability for productivity (h^2(P)) and quality (h^2(Q)) traits are compared in the figure. Lines connect average values of 400 simulation iterations of a given scenario, plotted with 95% confidence intervals. The illustrated comparison corresponds to a correlation among traits (r) equal to 0.1, equal economic weights on both traits ($a_1 = a_2 = 0.5$), and exponentially distributed induction success rates (E(RM) alternative).



spectively, while the increase of $V_{A(BP)}$ for the productivity trait was lower (because of lower h^2), yielding $V_{A(BP)}$ values of 167.3 and 57.1 at h^2 = 0.3 and 0.1, respectively. Inbreeding in the BP (F_{BP}) was zero in generations 1 and 2 and accumulated in later generations, reaching F_{BP} = 0.033 in generation 6 (census size of BP equal to 100) at the lower h^2 scenario and 0.043 at the higher h^2.

In the PP, the $V_{A(PP)}$ at the higher h^2 was over double that of lower h^2 ($V_{A(PP)} \approx$ 80–90 vs. $V_{A(PP)} \approx$ 30–40) (data not shown). There was no apparent change in $V_{A(PP)}$ over generations. F_{PP} in generation 6 was 0.064 and 0.082 for lower and higher h^2, respectively.

The effect of genetic correlation (r) between productivity and quality traits on the PP additive genetic response (A) and status number (N_S) is presented in Fig. 5. The scenarios depicted in the figure are for equal economic weights applied to both traits. Generally, r did not significantly affect the PP N_S (N_S is not shown in the figure), although some fluctuation was observed, particularly in earlier generations. At high r, the residual difference in responses of both traits was due only to the difference in their heritabilities. F_{PP} in generation 6 was between 0.07 and 0.085 at different r values (data not shown), but these differences were not statistically significant ($p > 0.05$).

Finally, the impact of economic weights on PP is demonstrated in Fig. 6, where r is kept constant at r = 0.1. The response in the quality trait is much larger compared to that of the productivity trait, as a result of higher h^2. In the majority of cases, the change in economic weights did not significantly alter N_S and F_{PP} (data not shown).

Discussion

Of great interest to those involved in somatic embryogenesis development and deployment is that variation in embryogenic propensity (i.e., induction success rates) has no impact on potential genetic gains in deployed clonal mixtures. The clonal deployment program is based on controlled crosses among elite individuals in the BP. In general, these individuals represent one extreme of a distribution of aggregate index breeding values, and the variance among them can be considerably lower than that of the entire BP. When a truncated normal distribution is considered, the phenotypic variance of the top 10% of BP represents only about 17% of the phenotypic variance of the entire BP, and consequently the additive genetic variance of the selected parents ranges between 17% and 100% of the additive variance of the BP, depending on h^2 (the "Bulmer effect", Bulmer 1985). Since these top individuals in the BP were selected based on their index value, which was calculated from the average clonal performance (average of 10 ramets) assessed in both traits, a greater reduction of additive variance is expected compared to the case when the assessment would be performed on a single phenotypic observation. The expected breeding value of an individual is the average of its parental breeding values (Falconer and Mackay 1996). The actual value of an offspring deviates from this expectation because of random selection of copies of parental genes (Mendelian sampling), giving rise to within-family variance. Thus, even though there is a reduced variation among family means by focusing on only the top 10% of BP, the assumed contribution of Mendelian sampling within each family is unchanged, depending only on the additive genetic variance in the unselected population and inbreeding of the parents. Therefore, it

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Fig. 5. Additive genetic response in the productivity and quality traits for the studied range of genetic correlation in the production population in generations 1 (Gen. 1) and 6 (Gen. 6). The figure corresponds to the heritability of productivity $h^2(P) = 0.3$ and the heritability of quality $h^2(Q) = 0.6$. Equal economic weights on both traits ($a_1 = a_2 = 0.5$) and exponential distribution of embryogenic induction success rates were assumed in the presented scenarios (E(RM) alternative).

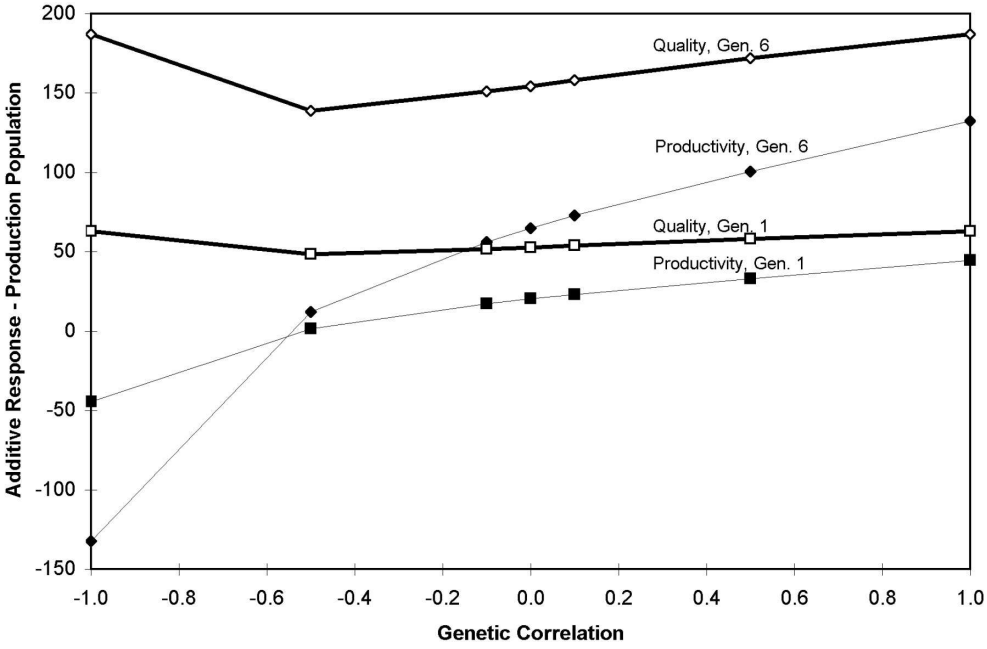
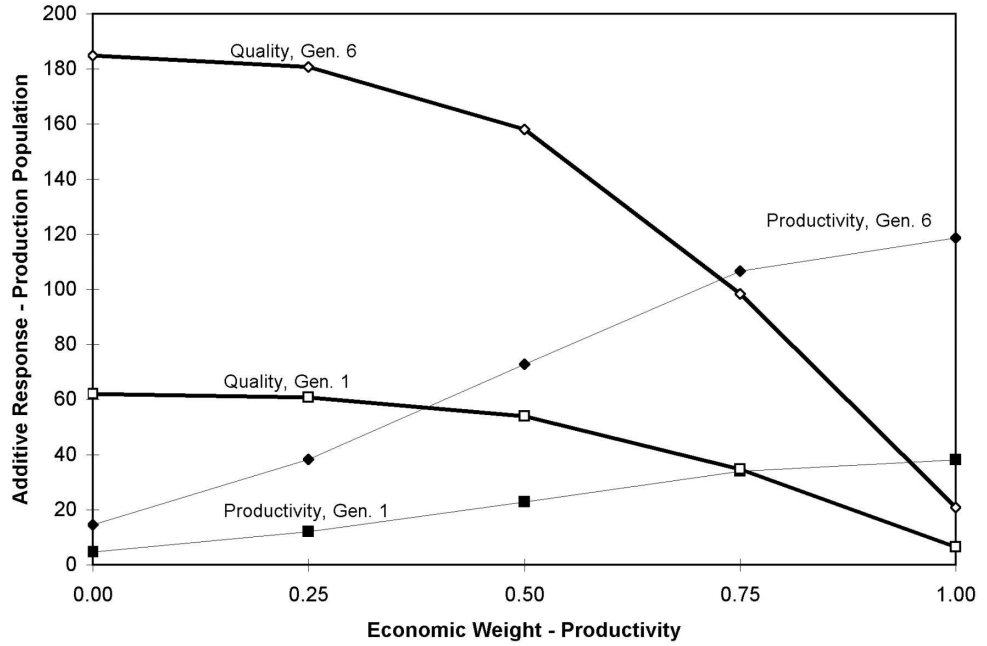


Fig. 6. Additive genetic response at different economic weights on productivity trait (a_1) in the production population in generations 1 (Gen. 1) and 6 (Gen. 6). The economic weight on quality (a_2) is equal to $1 - a_1$. Results correspond to heritabilities of 0.3 and 0.6 for productivity and quality traits, respectively. Correlation among traits (r) equal to 0.1 and exponential distribution of embryogenic induction success rates (E(RM) alternative) were assumed in the presented scenarios.



is possible to find superior genotypes for selecting a clonal mixture for deployment, provided that a proportion of families is highly successful in induction and cloning and that a large number of genotypes in these families are tested.

The previous argument notwithstanding, there remains some motivation for sharing the test effort among a larger number of families (rather than focusing on only the few where embryogenic induction rates are high). Maintenance of genetic diversity imposes a restriction on the maximization of genetic gain in the deployed clonal plantations. In reality, this dilemma has often been resolved by deploying a certain number of clones (rather than single) and expecting

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that the diversity of such a mixture would provide enough protection against plantation failure due to various catastrophic events (Sutton 2002). Based on the results in this study, it can be inferred that the effective population size of a periodically revised selected clonal mixture may fluctuate greatly from one time to another, even though the same census number of clones is selected each time. The solution to this can be to select with the goal to maximize genetic gain, but to restrict the outcome of such selection by a minimum effective population size, specified by the forest manager. Group-merit selection (Lindgren and Mullin 1997) can be used to provide such a solution. Maintaining larger numbers of families and clones in the test may be useful for two reasons: first, it would provide more choices for performing such selection; second, as the clonal test matures, the rank among clones in the combined index value may change considerably (economic weights in the index may change as well) and more opportunities would be available for the future.

Somatic embryogenesis technology has evolved considerably over the years. Merkle and Dean (2000) point to the shortcoming of this technology as the "low frequency of regeneration for many of the most desirable clones". Based on our simulation study, we believe that this problem may not be as severe as it might first appear. As somatic embryogenesis technology develops further, the normalization of responses among genotypes in individual steps is expected (Klimaszewska and Cyr 2002). The results of this study indicate that following such normalization and redirecting resources according to expected family values (as shown in Fig. 3, lines "E(BV)") would not necessarily lead to an important increase in genetic gains, if the testing effort is fixed. A positive effect of such redirection might appear under a much lower test budget than that considered here, when the average family size is considerably reduced and the variation among family means becomes more influential.

Higher values of h^2 for both traits were reflected in larger genetic gains in the BP. Since balanced within-family selection was practiced for population advancement, higher h^2 did not influence the effective population size of the BP. However, when a clonal mixture was selected (with no restrictions on relatedness), higher h^2 led to greater accuracy of selection among families, resulting in lower effective population size in the PP. In this model, h^2 of the quality trait was set to double that of the productivity trait. Even under slight correlation ($r = 0.1$), selection that focused primarily on productivity resulted in a substantial correlated response in quality, attributable primarily to the effect of higher h^2 of the quality trait. Some alternative methods of combining the value of both traits may be considered because of the observed sensitivity of responses to h^2, genetic correlation, and economic weights. An example could be the desired gain index, where instead of using economic weights the amount of desired response is specified for each trait, relative to each other (Pesek and Baker 1969; Harville 1975).

The breeding scheme (as modeled here) can be regarded as a single subline. A higher number of unrelated sublines might be established under real settings. Clonal mixtures could then originate from multiple sublines, making the effective population size of deployed mixtures larger than those evaluated here. Only additive gene action was considered in this study. In the presence of dominance interaction, inbreeding depression would become an additional factor. While we cannot quantify the effect of this factor using our model, we believe that it would not significantly change the main findings of this study.

The main conclusion of this study, that is, no significant impact of variation in embryogenic success on genetic gain in selected clonal mixtures, is of immediate interest to the forest industry. Normalization of responses among genotypes under constant budget may not necessarily lead to higher genetic response in selected clonal mixtures, as the majority of genetic variation in elite crosses was found to be within families. This conclusion holds for quantitative traits with arbitrary magnitudes of genetic correlation.

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