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Initiation of somatic embryogenesis in white spruce (*Picea glauca*): genetic control, culture treatment effects, and implications for tree breeding

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Abstract. The degree of genetic control and the effects of cultural treatments on somatic embryogenesis (SE) in white spruce were investigated with material derived from six-parent diallel crosses, including reciprocals. Thirty zygotic embryos from both immature and mature cones of each family were cultured in media with either 2,4-D or Picloram immediately after the collection of cones and after 2 months of cold storage. There were significant differences in SE initiation between immature and mature explants, and fresh and cold-stored seeds, but there was no significant differences with culture media effect. Significant variances due to families and to family × treatment interactions were found. The mean percentage of explants that initiated SE in each family ranged from 3.3% to 54.6%, with an overall average of 30.5%. The partitioning of family variance revealed that 21.7% was due to general combining ability effects, 3.5% was due to maternal effects, and 5.5% was due to reciprocal effects, but that the specific combining ability (SCA) was negligible. Variance due to interactions of family × treatments collectively accounted for 32.6%, while the remaining 37.8% of variation was accounted for by random error. However, when comparing the responses obtained with the treatment combinations, the SE response for freshly excised immature embryo explants showed comparatively large SCA variance, whereas the SCA variance was very small in the other treatment combinations.

Key words: Somatic embryogenesis – Clonal propagation – Genetic variances – Cultural effects – Tree breeding strategy

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Introduction

Somatic embryogenesis (SE) was first reported for Norway spruce (*Picea abies* Karst.) (Hakman et al. 1985; Chalupa 1985) and European larch (*Larix decidua* Mill.) (Nagmani and Bonga 1985) and has since been achieved for several other conifers (Cheliak and Rogers 1990; Tautorus et al. 1991). Plants derived from somatic embryos, called "emblings" (Libby 1986), are now routinely obtained, especially for *Picea* and *Larix* species. There are three distinct developmental stages in SE: initiation of embryogenic tissue masses, maturation of somatic embryos, and germination of mature somatic embryos. Each stage requires different culture treatments.

Effective SE techniques have important implications in tree improvement programs. First, they provide the means to mass-multiply material that has been genetically improved by breeding. Second, field testing of clones can be carried out over a long period because part of each clone can be maintained in a juvenile state by the cryopreservation of embryogenic cultures while the field test is in progress. This allows the use of "breeding-cloning" strategies (breeding followed by clonal multiplication for reforestation) instead of conventional seed orchard breeding practices (Mullin and Park 1992). There are many advantages in such strategies (Libby and Rauter 1984; Libby 1990), including the opportunity to capture both additive and non-additive genetic variances. Thus, genetic gains from breeding-cloning strategies are expected to be larger than those from conventional breeding programs.

Traditional means of producing clonal propagules, i.e., the rooting of cuttings, is limited by several factors. The most serious one is our inability to propagate individuals at a reasonable production rate and cost, mostly because of rapid maturation of the donor plants (ortets).

For example, in black spruce (*Picea mariana* (Mill.) B.S.P.), true-to-type propagation by the rooting of cuttings is possible only with young seedlings, and, therefore, an operational clonal program requires several cycles of serial propagation to meet stock requirements (Mullin and Park 1992; Mullin et al. 1992). Another problem is that our inability to maintain ortets in a juvenile state for long periods precludes long-term field testing of ramets and re-use of ortets after the tests have shown which ortets are the best. Hedging will extend juvenility in the ortets, but generally not long enough to allow the testing of clones for more than a few years.

SE is not yet used commercially in conjunction with breeding programs or in the mass production of propagules. It is still labor intensive and thus expensive. However, because the process is amenable to automation, it is expected that it will eventually become more economical than other clonal propagation techniques (Park and Bonga 1993). In addition, it still remains to be established that emblings will perform true-to-type in long-term field tests, i.e., that they do not mature prematurely, as is often the case with conifer clones obtained by organogenesis (Anderson et al. 1992). Initial results with field-tested emblings have been promising and, in the short-term at least, emblings have been shown to resemble seedlings (Webster et al. 1990, Roberts et al. 1991).

Our experiments had two main objectives. The first was to determine the degree of genetic control and genotype × treatment interactions in SE. The second was to examine how SE initiation was affected by the developmental stage (immature and mature) of the zygotic embryo explants, preconditioning of the explants by cold storage, and culture media. The primary focus was on the initiation of SE.

Materials and methods

Plant material and culture treatment

The material for the experiment was obtained in 1991 using 30 full-sib families derived from six-parent complete diallel crosses, including reciprocals but without selfs. The parents used in the controlled crosses were selected white spruce (*Picea glauca* (Moench) Voss) from a breeding population growing in a clone bank of the New Brunswick Tree Improvement Council. Both immature (early cotyledonary stage) and mature embryo explants excised from seeds obtained from the crosses were used. Half of the immature and mature seed cones were cold stored in paper bags at 4 °C for 2 months before the explants were excised.

The LM medium (Tremblay 1990) was used with half the plates containing 10 μmol 2,4-D and the other half containing 10 μmol Picloram (4-amino-3,5,6-trichloropicolinic acid) as auxin. Thirty explants, i.e., three plates with ten explants each, per family per treatment were cultured on each of the two media. The plates were sealed with Parafilm™ and placed in black plastic boxes inside a dark incubator that was kept at a constant 24.5 °C.

After about 2 weeks in culture, a light-brown tissue started to appear on most explants. After about 4 weeks, parts of this tissue started to produce translucent early embryogenic tissue. The explants were transferred to fresh initiation media after the initial 4 weeks and biweekly thereafter. After 16 weeks, the explants that still had not produced translucent and relatively necrotic-free embryogenic tissue were labelled as being non-embryogenic. As soon as embryogenic tissue was noticed, each explant with embryogenic tissue was transferred to a separate plate with fresh initiation medium (each subculture plate contained the tissue (clone) from one explant only). Subsequently, the plates were checked every 2 weeks. During each check, tissues large enough to be subdivided were cut into about 5 × 5-mm pieces and were transferred to fresh medium. The translucent parts of the tissue were preferentially selected for subculture if there was enough of it to do so. The most necrotic parts of the tissue were discarded. Some clones started growing rapidly immediately after the first subculture. After a few subcultures, these clones were subdivided into ten pieces of necrosis-free tissue per plate. In each subsequent subculture, this number of ten pieces per clone per plate was maintained. Excess pieces were discarded. These clones multiply their biomass 2–3 times between each transfer. These fast-growing, translucent, non-necrotic cultures were embryogenic and are labelled as such in our discussion of the results.

Statistical models and analyses

The embryogenic responses, expressed as percentages, were subjected to analysis of variance using the model:

$$Y_{ijkl} = \mu + F_i + M_j + S_k + R_l + FM_{ij} + FS_{ik} + FR_{il} + MS_{jk} + MR_{jl} + SR_{kl} + e_{ijkl} \quad (1)$$

where Y_{ijkl} is the percentage of embryogenic responses of the j-th maturation state explants of the i-th full-sib family with the k-th preconditioning storage placed in the l-th culture medium; μ is the experimental mean; F_i is the effect of the i-th full-sib family, $i = 1, \ldots, 30$; M_j is the effect of the j-th maturation state of the explant (mature or immature), $j = 1, 2$; S_k is the effect of the k-th preconditioning (fresh or cold stored), $k = 1, 2$; R_l is the effect of the l-th culture medium (2,4-D or Picloram), $l = 1, 2$; FM_{ij} is the interaction effect of the i-th family and the j-th maturation state; FS_{ik} is the interaction effect of the i-th family and the k-th preconditioning; FR_{il} is the interaction effect of the i-th family and the l-th culture medium; MS_{jk} is the interaction effect of the j-th maturation state and the k-th preconditioning; MR_{jl} is the interaction effect of the j-th maturation state and the l-th culture medium; SR_{kl} is the interaction effect of the k-th preconditioning and the i-th culture medium; and e_{ijkl} is the random error component.

The family term (F_i) was considered to be the random effect, and all of the other main effect terms in the model were considered to be fixed effects. The model did not include the second and higher-order interaction terms because these effects are often small and difficult to use in tree breeding. Consequently, the higher-order interactions were included in the error term. Computations of the analysis of variance were performed using a computer program, RUMMAGE (Bryce 1980), including expected means squares. Sum of squares due to family effects were further partitioned using the linear model:

$$Y_{mnh} = \mu + g_m + g_n + s_{mn} + m_m + m'_n + r_{mn} + E_{mnh} \quad (2)$$

where Y_{mnh} is the h-th observation of embryogenic response of the cross between m-th and the n-th parents; μ is the experimental mean; g_m (g_n) is the general combining ability effect of the m-th female (n-th male) parent, m, $n = 1, \ldots, 6$; s_{mn} is the specific combining ability effect for the cross between the m-th and the n-th parents, where $s_{mn} = s_{nm}$; m_m is the maternal effect.

Table 1. Form of the combined analysis of variance with expected mean squares

Source	*Df*	Expected mean squares
Family (F)	29	$\sigma_e^2+0.1\,\sigma_{FR}^2+0.1\,\sigma_{FS}^2+0.5\,\sigma_{FM}^2+0.1\,\phi_R+0.1\,\phi_S+0.4\,\phi_M+6.5\,\sigma_F^2$
GCA	5	$\sigma_E^2+0.7\,\sigma_{REC}^2+1.8\,\sigma_{MAT}^2+13.2\,\sigma_{SCA}^2+52.1\,\sigma_{GCA}^2$
SCA	9	$\sigma_E^2+0.7\,\sigma_{REC}^2+1.1\,\sigma_{MAT}^2+12.9\,\sigma_{SCA}^2$
MAT	5	$\sigma_E^2+12.7\,\sigma_{REC}^2+74.1\,\sigma_{MAT}^2$
REC	10	$\sigma_E^2+12.2\,\sigma_{REC}^2$
Maturity (M)	1	$\sigma_e^2+0.3\,\phi_{MS}+0.1\,\sigma_{FS}^2+3.7\,\sigma_{FM}^2+84.7\,\phi_M$
Storage (S)	1	$\sigma_e^2+0.3\,\phi_{MS}+3.5\,\sigma_{FS}^2+0.1\,\sigma_{FM}^2+94.5\,\phi_S$
Media (R)	1	$\sigma_e^2+0.2\,\phi_{SR}+0.3\,\phi_{MR}+3.5\,\sigma_{FR}^2+96.3\,\phi_R$
F × M	23[a]	$\sigma_e^2+0.1\,\phi_{MS}+0.1\,\sigma_{FS}^2+3.5\,\sigma_{FM}^2$
F × S	29	$\sigma_e^2+0.1\,\phi_{MS}+0.1\,\sigma_{FR}^2+3.1\,\sigma_{FS}^2$
F × R	29	$\sigma_e^2+0.1\,\phi_{SR}+0.2\,\phi_{MR}+0.1\,\phi_{MS}+3.1\,\sigma_{FR}^2$
M × S	1	$\sigma_e^2+35.5\,\phi_{MS}$
M × R	1	$\sigma_e^2+39.5\,\phi_{MR}$
S × R	1	$\sigma_e^2+43.5\,\phi_{SR}$
Error	79	σ_e^2

σ_e^2, σ_{FR}^2, σ_{FS}^2, σ_{FM}^2, and σ_F^2 are variance components due to error, family × media, family × storage, family × maturity, and family, respectively. ϕ_{SR}, ϕ_{MR}, ϕ_{MS}, ϕ_R, ϕ_S and ϕ_M are variances due to fixed effects of storage × media, maturity × media, maturity × storage, media, storage, and maturity, respectively. For example, ϕ_M is the quantity $\Sigma_j M_j^2$. The component σ_E^2 is the residual error variance due to fitting model 2, which includes all sources of variance in model 1 except σ_F^2. The components, σ_{REC}^2, σ_{MAT}^2, σ_{SCA}^2, and σ_{GCA}^2, are variances due to reciprocal, maternal, specific combining ability, and general combining ability, respectively

[a] Six degrees of freedom were lost due to imbalance in the data

of m-th female line, where $m'_n = -m_n$; r_{mn} is the reciprocal effect involving the reciprocal crosses of the m-th and the n-th parents, where $r_{mn} = -r_{nm}$; E_{mnh} is the residual error component.

All of the terms in the model, except experimental mean, were considered to be random effects, and computations, including expected mean squares, were carried out using a computer program, DIALL (Schaffer and Usanis 1969). The form of analysis of variance, combined over the two linear models (1 and 2) is presented in Table 1. For all random effect terms, variance components were estimated and the standard deviation of the estimates were calculated following Anderson and Bancroft (1952):

$$\sqrt{\sum_i [2\,k_i^2\,(MS_i)^2/(df_i+2)]}$$

where k_i is the coefficient of the linear combination of mean squares, MS_i is the mean square used to estimate the component, and df_i is the respective degree of freedom for the mean square.

The significance tests were performed using approximate F-tests by comparing expected mean squares (Satterthwaite 1946; Tietjen 1974) at the 5% probability level. Once significant family × treatment interactions were found, based on linear model 1, separate analyses of variance were performed using model 2 for each of the four treatment combinations, i.e., freshly excised immature (FI), cold-stored immature (SI), freshly excised mature (FM), and cold-stored mature (SM) embryo explants.

The percentage data were transformed by using arcsine values of the square roots of percent data ($\sin^{-1}\sqrt{\%}$) for all analyses of variance; however, the means are based on the actual percentages (Sokal and Rohlf 1969).

Results and discussion

Effects of cultural treatment

The data from the experiment are summarized in Table 2. The percentages of cultures that formed light-brown tissue masses (TM) are based on the initial total number of explants. If 30 explants had been used for each family and each treatment, the experiment would have been carried out with a total of 7,200 explants (30 explants × 30 families × 2 maturity states × 2 storage conditions × 2 culture media). In some cases, due to non-availability, slightly fewer than 30 explants were used per family per treatment, resulting in a total of 5,572 explants being used. The percentages of explants forming embryogenic (ET) and non-embryogenic tissue masses (NET) are based on the number of explants that were neither dead nor contaminated after 16 weeks. The reasons for this adjustment are given later in this paper. After 16 weeks of culture, 85.2% of the explants had formed the initial light-brown tissue mass (TM). Of these, 32.9% eventually formed the translucent embryogenic tissue mass (ET). The percentage of contaminated (CONT) and dead (DEAD) cultures was small, 2.5 and 12.2%, respectively.

The maturation state of the explants had a significant effect on TM, ET, NET, and the percentage of explants that died (DEAD). TM was higher for mature (95.0%) than for immature (74.0%) explants, but ET was more than twice as high for the immature than for the mature explants (46.8% vs 20.8%). DEAD was more than 10 times higher for the immature embryo explants than for the mature ones (23.5% vs. 2.4%). This could in part be the result of the immature explant being smaller and more fragile than the mature one. It is also possible that some of the explants were still too immature when excised, i.e., they had not yet reached the stage of development at which they are capable of forming embryogenic and non-embryogenic tissue masses. On the basis of these considerations, we decided that ET and NET in Table 2 should be based on the number of explants that had produced cultures surviving after 16 weeks of culture rather than on the total number inoculated.

Fresh and cold-stored explants behaved differently in vitro. A significantly higher percentage of the explants excised from fresh material immediately after cone collection than of explants excised from cold-stored cones produced TM (88.4% vs 81.5%) and ET (38.5 vs 26.4%). The percentages of CONT and DEAD explants were

Table 2. Mean percentage (standard error) of cultures that formed a light-brown tissue mass (TM), embryogenic tissue (ET), and non-embryogenic tissue (NET), and became contaminated (CONT) or dead (DEAD) after 16 weeks of culture

Treatments	TM[b]	ET[d]	NET[d]	CONT	DEAD
Maturity					
Immature	74.0(2.0)A[c]	46.8(2.3)A	53.2(2.3)A	2.5(0.7)	23.5(1.9)A
Mature	95.0(0.9)B	20.8(1.5)B	79.2(1.5)B	2.5(0.8)	2.5(0.4)B
Storage					
Fresh	88.4(1.5)A	38.5(2.4)A	61.5(2.4)A	1.5(0.6)A	10.1(1.4)A
Cold-stored	81.5(2.1)B	26.4(2.0)B	73.6(2.0)B	3.7(0.9)B	14.8(1.9)B
Media					
2,4-D	83.7(1.9)	31.4(2.2)	68.6(2.2)	3.1(0.9)	13.2(1.7)
Picloram	86.9(1.7)	34.5(2.4)	65.5(2.4)	1.9(0.6)	11.2(1.6)
Mean:	85.2(1.3)	32.9(1.6)	67.1(1.6)	2.5(0.5)	12.2(1.2)
Range[a]:	25.0–100.0	0.0–94.4	5.5–100.0	0.0–42.9	0.0–75.0

[a] Means based on 30 explants per family per treatment, except for ET and NET where the number may be slightly lower due to adjustment (see [d])
[b] Percentage of explants with a live tissue mass (TM) before any adjustment, where TM + CONT + DEAD = 100%
[c] Different letters between the pairs of means indicate significant differences at the 5% level
[d] Adjusted for contaminated and dead explants (see text), where ET + NET = 100%

lower in the freshly excised material than in the cold-stored material (1.5% vs 3.7%, and 10.0% vs 14.8%, respectively).

Whether the medium contained 2,4-D or Picloram did not make a significant difference. The percentage of explants forming embryogenic tissue was slightly, but not statistically, higher with Picloram in the medium.

The percentages of explants forming embryogenic tissue over time are shown in Fig. 1. These percentages are based on the total number of explants inoculated. They were not based on the total number of explants minus the contaminated and dead ones because death was not recorded before the 16th week. The highest percentages of explants forming embryogenic tissue were obtained consistently with fresh, immature explants. At the 16th week, 42.8% of these explants had formed embryogenic tissue. This was followed in descending order by cold-stored immature (25.3%), fresh mature (25.1%), and cold-stored mature (15.4%) explants.

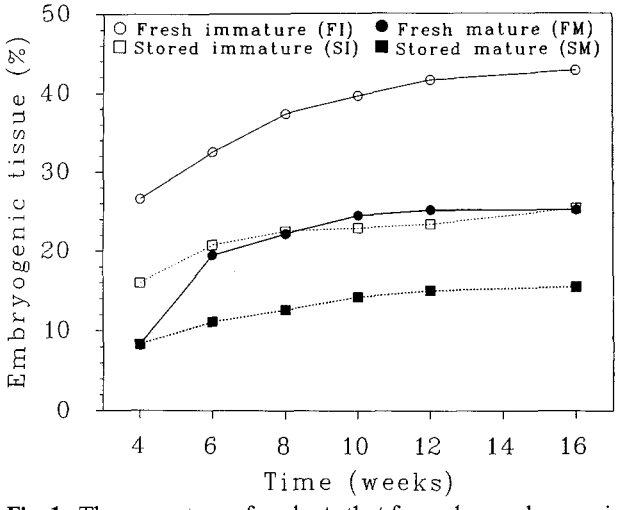

Fig. 1. The percentage of explants that formed an embryogenic tissue mass over the 16-week period for each treatment combination

Genetic effects

The variance components due to families were significant for all characters examined in Table 2, except for the percentage of cultures that became contaminated. However, our further discussion on genetic effects will deal only with the initiation of embryogenic tissue. Estimated variance components from a combined analysis of variance are presented in Table 3. The variance component due to the general combining ability (σ^2_{GCA}) was the largest source of genetic variance, amounting to 21.7% of the total variance. The variance due to the maternal (σ^2_{MAT}) and the reciprocal (σ^2_{REC}) effects accounted for 3.5% and 5.5% of the total variance, respectively, while that due to the specific combining ability (σ^2_{SCA}) was negligible. Thus, a total of 30.7% of the variance was due to genetic effects. Error variance accounted for 37.8% of the total variance.

Interpretation of the genetic components of variance requires the usual assumptions, as described by Cockerham (1963), i.e., parents are random samples taken from a panmictic population, there is a regular diploid Mendelian inheritance and linkage equilibrium, and there are no

Table 3. Estimated variance components (standard deviation) and the percentage of total variance for initiation of embryogenic tissue

Variance component	Estimate	Percentage
σ^2_{GCA}	42.6(24.0)*	21.7
σ^2_{SCA}	− 7.2(4.0)	0.0
σ^2_{MAT}	6.9(6.3)	3.5
σ^2_{REC}	10.7(10.8)	5.5
σ^2_{FM}	29.2(14.9)*	14.9
σ^2_{FS}	32.8(14.9)*	16.7
σ^2_{FR}	− 8.0(5.6)	0.0
σ^2_e	74.4(11.7)	37.8
Total	196.6	100.0

* Significant at $P \leq 0.05$

Table 4. Mean percentage of explants that formed embryogenic tissue by each full-sib family

Female	Male					
	DNR-27	GPC-02	MRL-03	GPC-03	GPC-15	MRL-11
DNR-27		22.8	42.9	24.9	38.5	43.9
GPC-02	23.2		27.9	3.8	15.0	32.2
MLR-03	39.7	17.0		42.2	45.5	39.8
GPC-03	54.1	22.0	47.0		46.1	40.0
GPC-15	22.6	3.3	43.4	8.9		44.9
MRL-11	21.1	15.2	54.6	35.6	24.1	
$\hat{g}_m$	1.6	−11.0	7.2	0.9	−1.8	3.1

Parents are labelled according to the accession code for the white spruce breeding population of the New Brunswick Tree Improvement Council, a cooperative tree improvement program. $\hat{g}_m$ is the general combining ability value (Griffing 1956) if the parents are assumed to be a fixed set

environmental correlations among relatives. The general combining ability (GCA) variance is the variance due to the average effect of parents and is translated into covariance of half-sibs. For a population derived from non-inbred parents, the covariance of half-sibs represents one-quarter of the additive genetic variance (σ^2_A). Thus, the additive genetic variance is estimated by multiplying σ^2_{GCA} by 4. The specific combining ability (SCA) variance is translated into covariance of full-sibs after the maternal and paternal covariances of half-sibs are subtracted. This represents one-quarter of the dominance genetic variance (σ^2_D). Since the estimate for σ^2_{SCA} in this experiment was negative, it was considered to be zero. The maternal component of variance (σ^2_{MAT}) is the variation due to the average maternal effect of each parent. The reciprocal component of variance (σ^2_{REC}) is the variation due to the reciprocal differences not accounted for by σ^2_{MAT}. Although, in our experiment both σ^2_{MAT} and σ^2_{REC} were present, the magnitude of these components was small compared to σ^2_{GCA}. Therefore, the initiation of SE in white spruce is under strong additive genetic control. The narrow-sense heritability, defined as the ratio of additive genetic variance to total phenotypic variance (σ^2_{PH}), was 0.71, where $\sigma^2_{PH} = 2\sigma^2_{GCA} + \sigma^2_{MAT} + \sigma^2_{REC} + \sigma^2_{FM} + \sigma^2_{FS} + \sigma^2_e$. This estimate is high compared to the estimates available for many metric traits of forest tree species and indicates that it will be relatively effective to select for SE in breeding programs.

Since, in our analysis, the parents were considered to be random samples within a breeding population (random effect), we did not carry out statistical comparisons of full-sib family means. It is, however, useful to examine the distribution of full-sib family means with respect to parentage. All of the full-sib families used in the experiment became embryogenic with at least one of the treatments, and the percentage of SE was as high as 94.6% (Table 4, FI explants of cross GPC-03 × GPC-15 cultured with Picloram). The mean percentages of cultures that produced embryogenic tissue ranged from 3.3% to 54.6% with an overall mean of 26.2% (Table 4). The average additive contribution of each parent, expressed as the deviation from the overall mean, ranged from −11.0% to 7.2%. These are the general combining ability values calculated, as described by Griffing (1956), if the parents are assumed to comprise a fixed set of breeding material. For example, the use of MRL-03 as a parent is expected to increase the percentages of explants forming embroygenic tissue by an average of 7.2% above the overall mean, while the average contribution of GPC-02 is expected to be 11.0% below the overall mean.

The genetics of plant regeneration in vitro is complex (Carman 1990). In detailed studies with wheat (*Triticum aestivum* L.) Kaleikau et al. (1989) found that regeneration from immature embryo explants is genotype dependent. Analyses of crosses between highly and poorly regenerative genotypes indicated a qualitative mode of inheritance, with one or possibly two dominant genes being prominent in some crosses, whereas in other crosses major genes and additional modifier genes appear to be involved. SE in cultures of rice (*Oryza sativa* L.) appears to be under the control of nuclear genes and involves additive and dominance effects as well as cytoplasmic genes that cause reciprocal effects (Peng and Hodges 1989). This has also, been reported for wheat (Lazar et al. 1984). Willman et al. (1989), working with corn (*Zea maize* L.), found that, in addition to additive and dominance gene effects, there were also significant cytoplasmic and maternal and/or paternal effects, and suggested that at least one gene or a block of genes controls SE. In these studies, the additive gene effects were more important than all of the other genetic effects. Thus, genotypic specificity of plant regeneration appears to be common in cereal crop species. This also seems to be the case with conifer species, as the results of our experiment indicate. Tremblay (1990) reported that SE of white spruce was

significantly affected by the origin of the seed provenances. Cheliak and Klimaszewska (1991) found significant differences among open-pollinated families in SE of black spruce.

Genotype × treatment interactions

The variances due to family × maturity and to family × storage interactions were significantly large, accounting for 14.9% and 16.7% of the total variance, respectively; however, the family × media interaction was negligible (Table 3). Furthermore, the total variance due to interactions ($\sigma^2_{FM}+\sigma^2_{FS}$) was as large as the total of all the genetic components of variance ($\sigma^2_{GCA}+\sigma^2_{SCA}+\sigma^2_{MAT}+\sigma^2_{REC}$). Genotype × treatment interactions of this magnitude are likely to have an impact on selection, and this necessitates close examination at each treatment combination. Since neither the effect of culture media (2,4-D vs Picloram) nor family × media interaction were significant, we considered the culture media as the replicates in the separate analyses of variance for each treatment combination.

Estimated variance components are presented separately for each of the four treatment combinations in Table 5. Variance due to the GCA among the treatment combinations ranged from 10.3% to 44.7%. It was the largest genetic component of variance (i.e., σ^2_{GCA}, σ^2_{SCA}, σ^2_{MAT}, and σ^2_{REC}) among the treatment combinations except for FI, where σ^2_{MAT} was the largest. Variance due to the SCA ranged from 0.0% to 17.0% but, except for the FI combination, it was relatively small. This indicates that the embryogenic response is controlled by mainly additive genetic effects at least for SI, FM, and SM. Variance due to the maternal effect was the largest for FI (18.4%) and was negligible for FM. The reciprocal effect was also substantial among the treatment combinations, ranging from 12.6% to 21.4%. The percentage was practically the same (21%) for SI, FM, and SM; the percentage for FI (12.6%) was considerably lower.

The embryogenic response for FI was distinctly different from that of SI, FM, and SM, as characterized by the higher rate of response (Fig. 1), high SCA and maternal variances, and comparatively low GCA variance. The variance due to SCA declined dramatically from 17.0% for FI to 0.0% for SI (Table 5). The distinctly different SE response and the sudden decline of SCA variance when using SI, FM or SM explants instead of FI explants indicate that we are dealing with fairly drastic changes in activity in several genes during the storage and maturation of the zygotic embryo. Similar changes in gene activity during embryo development have been found by Li et al. (1992) for barley. They have isolated four embryo-specific cDNA clones, which represent four different expression patterns that change during embryo development and germination. During maturation of the zygotic embryo considerable changes occur in its biochemical characteristics, and this appears to correlate with the ability of embryo explants to initiate SE. For example, Roberts et al. (1989) found that the competence of white spruce zygotic embryos to initiate SE diminished rapidly after they reached the developmental stage where storage proteins started to accumulate. This again indicates a large change in genetic activity during embryo maturation.

As mentioned previously, genotype × treatment interactions have an impact on selective breeding because these interactions often result in changes in rankings of families (genotypes) over a range of treatments. Although statistically significant rank correlations were found between FI and FM ($r=0.42$), SI and SM ($r=0.44$), and FM and SM ($r=0.50$), changes in the rankings of family means occurred among treatment combinations.

The SE responses of all individual families over the treatment combinations were further examined by using a stability analysis based on a regression approach (Finlay and Wilkinson 1963; Eberhart and Russell 1966). The analysis involved computation of the linear regression

Table 5. Estimated variance components (standard deviation) for treatment combinations, i.e., freshly excised immature (FI), cold-stored immature (SI), freshly excised mature (FM), and cold-stored mature (SM) seed embryos. The percentage indicates the proportion of each variance component to total variance

Variance component	FI		SI		FM		SM		Mean
	Estimate	%	Estimate	%	Estimate	%	Estimate	%	%
σ^2_{GCA}	16.2(18.5)	10.3	56.3(33.9)*	32.8	62.3(35.3)*	44.7	26.4(17.9)*	22.7	27.6
σ^2_{SCA}	26.7(29.0)	17.0	−14.1(21.0)	0.0	0.7 (7.5)	0.5	2.9(13.7)	2.5	5.0
σ^2_{MAT}	29.0(20.9)	18.4	20.9(21.4)	12.2	−0.3 (5.4)	0.0	7.6 (9.9)	6.5	9.3
σ^2_{REC}	19.8(19.9)	12.6	36.6(32.3)*	21.3	29.8(18.8)*	21.4	24.4(20.2)*	21.0	19.1
σ^2_E	65.2(18.2)	41.7	58.0(18.4)	33.7	46.6(12.0)	33.4	55.1(15.6)	47.3	39.0
Total	156.9	100.0	171.8	100.0	139.4	100.0	116.4	100.0	100.0

* Significant at $P<0.05$

Table 6. Mean percentage of explants forming embryogenic tissue for full-sib families using fresh immature (FI), cold-stored immature (SI), fresh mature (FM), and cold-stored mature (SM) embryo explants, and their stability parameters. The means are averaged over the two media

Family		Treatment combinations					Stability parameters		
Female	Male	FI	SI	FM	SM	Mean[a]	Regression	Deviation[b]	R^2
DNR-27	GPC-02	24.0	43.9	5.0	18.5	22.9	0.41	324	0.17
DNR-27	MRL-03	47.3	56.0	40.0	28.3	42.9	0.52	99	0.52
DNR-27	GPC-03	50.8	12.5	20.7	9.4	23.3	0.99	152	0.72
DNR-27	GPC-15	60.4	47.0	17.6	29.2	38.5	1.03	124	0.77
DNR-27	MRL-11	66.7	60.8	30.0	18.3	44.0	1.37	87	0.89
GPC-02	DNR-27	49.2	11.1	9.3	–	23.2	1.46	146	0.86
GPC-02	MRL-03	72.5	20.0	7.0	8.4	27.0	1.76	222	0.84
GPC-02	GPC-03	–	0.0	7.0	2.5	3.2	−0.14	21	0.18
GPC-02	GPC-15	11.1	44.4	0.0	9.8	16.3	0.31	525	0.07
GPC-02	MRL-11	68.3	–	25.9	2.5	32.2	1.69	35	0.98
MRL-03	DNR-27	65.9	53.9	30.0	8.9	39.7	1.57	61	0.95
MRL-03	GPC-02	41.2	13.0	10.5	3.4	17.0	0.97	44	0.89
MRL-03	GPC-03	61.4	56.7	31.7	19.1	42.2	1.18	66	0.89
MRL-03	GPC-15	86.3	55.4	21.7	18.8	45.5	1.93	58	0.96
MRL-03	MRL-11	67.9	29.6	40.0	21.7	39.8	1.07	160	0.74
GPC-03	DNR-27	80.6	67.6	37.1	31.3	54.2	1.42	51	0.93
GPC-03	GPC-02	27.4	42.3	12.1	6.0	22.0	0.69	210	0.47
GPC-03	MRL-03	66.1	–	31.7	43.2	47.0	0.76	177	0.71
GPC-03	GPC-15	72.2	40.6	43.3	28.3	46.1	1.07	72	0.86
GPC-03	MRL-11	70.3	31.7	31.7	26.4	40.0	1.13	122	0.80
GPC-15	DNR-27	–	–	36.7	8.6	22.7	–	–	–
GPC-15	GPC-02	–	–	6.7	0.0	3.3	–	–	–
GPC-15	MRL-03	56.4	44.2	51.3	21.8	43.4	0.73	140	0.60
GPC-15	GPC-03	–	–	4.4	13.5	8.9	–	–	–
GPC-15	MRL-11	61.3	28.6	–	–	44.9	–	–	–
MRL-11	DNR-27	–	–	31.7	0.0	15.8	–	–	–
MRL-11	GPC-02	20.0	6.7	16.7	20.0	15.8	−0.04	59	0.01
MRL-11	MRL-03	51.7	36.4	66.7	–	51.6	−0.44	382	0.17
MRL-11	GPC-03	36.7	33.3	–	–	35.0	–	–	–
MRL-11	GPC-15	13.7	43.6	27.1	12.1	24.1	0.06	319	0.00
Mean:		53.1	36.6	24.8	15.8	30.5	0.89	158	0.62

–, Missing or insufficient data. The families with missing data on two treatment combinations were excluded from stability analysis.
[a] Averaged over treatment combinations
[b] Deviation mean squares

for each family onto a treatment index used as a set of independent variables. The treatment index consisted of the means for the treatment combinations over all the families. Thus, the index values for FI, SI, FM, and SM were 53.1, 36.6, 24.8, and 15.8%, respectively. These values represent the relative productivity of the treatment combinations (Table 6, Fig. 2). The stability of a family is then determined by two parameters: one, the regression coefficient that measures the linear response of families over a range of treatments and, two, the deviation mean square from the regression. Thus, a regression coefficient defines a predictable portion of the SE response, while the deviation measures unpredictable irregularities.

The SE response of each full-sib family at different treatment combinations, along with the stability parameters, are presented in Table 6. By definition, a stable genotype is one that performs the same over a range of treatments or environments, which implies a regression coefficient of zero. In plant breeding, however, low regression coefficients are often associated with low yield (Eberhart and Russell 1966), and a zero coefficient indicates that there is no linear relationship between yield and environment (Shukla 1972). These are negative features and, consequently, many authors prefer to use the average stability to define a stable genotype, i.e., having a regression coefficient of 1. Although the regression coefficient defines a predictable portion of a family, when the deviation mean square increases, the response of a family becomes less predictable and thus unstable. Stability parameters among the full-sib families were vari-

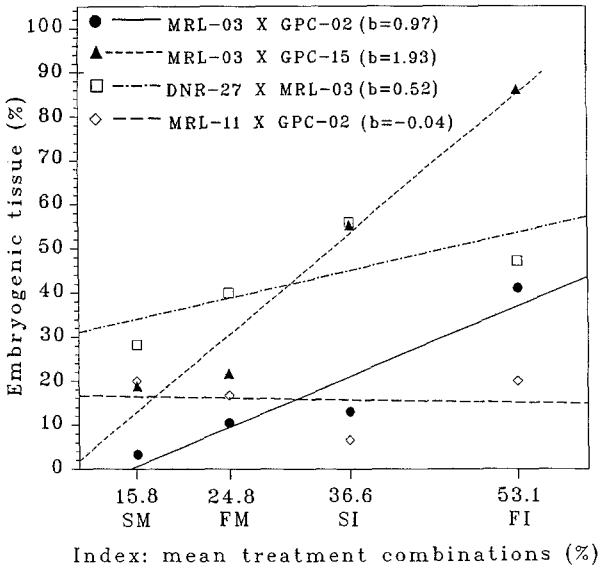

Fig. 2. Embryogenic response patterns, based on regression coefficient (b), for a sample of four full-sib families using a stability analysis (see text). The reference points on the X-axis indicate the means of the treatments, i.e., cold-stored mature (SM), fresh mature (FM), cold-stored immature (SI), and fresh immature (FI). Changes in rankings of family means over the range of treatments are demonstrated. For example, in SM the ranking from high to low was □, ◇, ▲, and ●, but in FI the ranking was ▲, □, ●, and ◇

able. It is noted however, that each of the families with MRL-03 as the female parent had relatively small deviation mean squares ranging from 44 to 160 (Table 6). Incidentally, MRL-03 was also the parent with the highest GCA value (Table 4). The families with MRL-11 as the female parent had low regression coefficients (Table 6).

To illustrate possible patterns of family responses over the treatment combinations, a sample of four full-sib families was plotted in Fig. 2. The family resulting from MRL-03 × GPC-02 had a regression coefficient close to 1 (0.97) with a low deviation mean square and, therefore, may be called a stable family (Table 6). The SE response of this family increased steadily as the treatment combination improved. Of the four families shown in Fig. 2, the response of MRL-03 × GPC-02 was the poorest at the poorest treatment, but it was about average at the best treatment combination. The family MRL-03 × GPC-15 had the highest regression coefficient (1.93) with a small deviation mean square. This family may be characterized as a highly responsive family when the treatment becomes favorable: at the poorest treatment combination, it was mediocre, but at the best treatment combination, the family had the highest percentage of explants with embryogenic tissue. On the other hand, the family MRL-11 × GPC-02 was non-responsive regardless of treatment combinations; its regression coefficient was close to zero (−0.04). The family DNR-27 × MRL-03 had a regression coefficient of less than 1 (0.52), which may be considered a below-average response over the range of treatment combinations.

Implications for tree breeding

Clonal propagation has long been recognized as a useful technique to be used together with tree breeding, primarily because of its potential for mass multiplication of genetically improved material. So far, the cloning of improved genetic material has been carried out by rooting the cuttings. However, SE promises to be a far more effective technique for achieving true-to-type clonal propagation on an operational level, at least for white spruce.

We have obtained embryogenic tissue from all of the full-sib families used in the experiment. This is encouraging because the ability to clone all or nearly all of the full-sib families obtained by breeding is essential to make "breeding-cloning" strategies effective. In addition, for many of the full-sib families, a high percentage of the explants became embryogenic. This too is important because the higher the number of responsive explants in each of the families, the more genotypes are available for selection. If SE provides only a few genotypes, a serious loss of genetic diversity would occur in the operational "breeding-cloning" programs. Our results also indicate that for some families the percentage of explants becoming embryogenic can be increased by the use of improved treatment combinations. The exclusive use of freshly excised immature embryos would improve the overall response of SE. In our experiment, the freshly excised immature embryos produced about 3.4 times more embryogenic cultures than the cold-stored mature embryos (53.1 vs 15.8%) (Table 6). Since the genetic variation in the embryogenic response in our material is under strong additive genetic control, selection and breeding for an increased embryogenic response is a practical option. It is, however, somewhat complicated by large variances due to genotype × treatment interactions. In operational breeding programs, selection for a high SE response is likely to be carried out with one treatment combination only, i.e., the one that, on average, produced the highest SE response, which in our case would be with fresh immature explants. In this case, the effectiveness of selection is comparatively lower than with the other treatment combinations due to a high SCA variance. However, this is more than offset by a moderately high narrow-sense heritability of 0.37, and genetic gain is, therefore, likely to be respectable. In addition, the existence of genotype × treatment interactions indicates that there is a possibility of increasing SE by prescribing specific treatments for some families. For example, there were a few cases where the SE response was not the highest when fresh immature explants were used. To im-

prove the initiation of SE in relatively recalcitrant families the modification of existing culture procedures may be required.

Vegetative propagation will have an important role in progeny testing. Shaw and Hood (1985) suggested that the use of clonal replicates in genetic testing would increase precision in the ranking of individuals with families for recurrent selection and thus increase the cumulative genetic gain obtained during each cycle of breeding. Also, to predict genetic gains from breeding-cloning strategies, it is necessary to estimate both additive and non-additive genetic variances. Progeny testing, using clonally replicated families, offers a possibility to obtain such estimates. For example, Park and Fowler (1987) estimated both additive and non-additive genetic variances from a progeny test of clonally replicated open-pollinated families of tamarack (*Larix laricina* (Du Roi) K. Koch). The use of clonal replicates from controlled-pollinated families can further resolve the non-additive variance due to dominance and epistatic gene effects (Mullin and Park 1992; Mullin et al. 1992). Most of the tree breeding literature assumes that non-additive variance is composed of dominance variances; therefore, there is a general lack of information on epistatic genetic variance of forest trees. A common method of estimating epistatic variance is to develop inbred lines so that exact genotypes are known (Hallauer and Miranda 1981; Mather and Jinks 1982), but this is impractical with forest trees. Mullin et al. (1992), using a clonally replicated progeny test, demonstrated that substantial epistatic variance exists in early growth of black spruce. Thus, effective vegetative propagation technology will help to resolve the genetic variance structure in progeny testing and will thus confirm the suitability of clonal breeding strategies. Here again SE will become important and probably replace the rooting of cuttings in these programs.

Tautorus et al. (1991) reviewed other potential benefits of conifer SE, which include long-term germ plasm storage and the provision of cells suited for formation of protoplasts to be used for genetic transformation and for somatic hybridization. It is expected that these benefits will eventually have a considerable impact on tree breeding and on our understanding of conifer genetics.

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