Clonal variation in morphology, growth, physiology, anatomy and ultrastructure of container-grown white spruce somatic plants

MOHAMMED S. LAMHAMEDI, HELÈNE CHAMBERLAND, PIERRE Y. BERNIER and FRANCINE M. TREMBLAY

1 Centre de Recherche en Biologie Forestière, Faculté de Foresterie et de Géomatique, Pavillon Charles-Eugène Marchand, Université Laval, Québec G1K 7P4, Canada
2 Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, 1055 du PEPS, P.O. Box 3800, Sainte-Foy, Québec G1V 4C7, Canada
3 Present address: Ministère des Ressources naturelles, Forêt Québec, Direction de la recherche forestière, 2700 rue Einstein, Sainte-Foy, Québec G1P 3W8, Canada
4 Author to whom correspondence should be addressed

Received April 7, 1999

Summary We assessed clonal variation in morphological variables, mineral nutrition, root growth capacity, net photosynthesis, tannin distribution, and cuticle and epicuticular wax features within four families of white spruce (Picea glauca (Moench) Voss). Seeds were collected from four families obtained through controlled crosses among selected genotypes. For each family, plants were produced either from seeds (zygotic) or by somatic embryogenesis (clones). Each family was therefore represented by its zygotic seedlings and three clones. Within a family and under similar growth conditions, several clones differed significantly from the zygotic seedlings in height, root-collar diameter, needle dry mass, branch density, shoot dry mass, root dry mass, and length of needles. Branch density (number of first-order branches per cm height) of zygotic seedlings and clones varied from 0.8 to 1.4 branches cm⁻¹ and from 0.6 to 1.3 branches cm⁻¹, respectively. Mean needle length of zygotic seedlings and clones ranged from 11 to 14 mm and from 11 to 17 mm, respectively. For many variables (height, dry mass of new roots, needle dry mass and branch density), differences among clones were significantly greater than differences among zygotic seedlings within a family. Tannins were more abundant in needles of clones than in needles of zygotic seedlings. In some clones, tannins occurred as a ribbon along the central vacuole, whereas in others they appeared as aggregates dispersed in the vacuole. Within a family, N, P and K showed considerable variations in their use efficiency. Interclonal variations were observed in root growth potential and net photosynthesis. Variations in growth and physiology reflect genetically determined differences among clones within a family.

Keywords: clones, mineral nutrition, net photosynthesis, Picea glauca, root growth potential, somatic embryogenesis, tannin distribution.

Introduction

White spruce (Picea glauca (Moench) Voss) is one of the most widely distributed tree species in Canada (Niensstaedt and Zasada 1990). In Québec, white spruce is used in artificial reforestation programs, with an annual production objective of 33 million seedlings in the year 2000 (Daoust and Beaulieu 1993). A breeding program was initiated several years ago to complement this regeneration program. Breeding strategies using vegetative propagation to produce improved clonal stock are being promoted as an alternative to conventional seed-orchard procedures, and several studies have shown the potential of including somatic embryos of white spruce in breeding programs (Tremblay 1990, Park et al. 1993, 1994). For example, somatic embryogenesis (SE) can be used to propagate superior genotypes rapidly and with great efficiency (Tautorus et al. 1991). Clonal propagation through SE can also enhance genetic gains (Libby and Rauter 1984, Libby 1986). In addition, SE will facilitate genetic manipulations such as the introduction of Bacillus thuringiensis toxin genes into embryogenic cells of white spruce to regenerate plants resistant to the spruce budworm (Séguin et al. 1993, Li et al. 1994, Séguin 1999).

However, before SE can be introduced into forest regeneration programs, we need to determine the physiological and morphological attributes of somatic plants and to assess how these attributes compare with those of normal zygotic seedlings of the same families. Evaluation of seedling quality, through material and performance attributes, provides a means to predict seedling survival and growth after planting. Although several recent studies (Grossnickle and Major 1994, Grossnickle et al. 1994) compared somatic and zygotic seedlings of interior spruce (Picea glauca (Moench) Voss × Picea engelmannii Parry ex Engelm.) in terms of morphological and physiological variables, these studies did not examine interclonal variation. Our hypothesis was that morphological and
physiological variables differ among clones within the same family and that these differences are under genetic control. Therefore, the first objective was to compare the variability in selected attributes of white spruce clones and zygotic seedlings from the same family. The second objective was to characterize clones derived from SE for potential use in reforestation programs.

To achieve these objectives, somatic and zygotic seedlings were compared in terms of histological, morphological, and physiological variables, particularly the physiological variables linked to field performance. For boreal species, nutrient status affects field performance, which is defined as survival and growth after planting, by influencing several physiological factors such as frost hardiness, root growth potential, and drought tolerance (Landis 1985, Munson and Bernier 1993, Lamhamedi and Bernier 1994, McAlister and Timmer 1998). For many species, root growth potential (RGP)—the ability to regenerate new roots—is closely linked to field performance (Feret and Kreh 1985, Larsen et al. 1986, Simpson and Ritchie 1997). Net photosynthesis has also been used successfully to evaluate seedling quality under controlled environments (Stewart and Bernier 1995, Lamhamedi et al. 1997) and field conditions (Grossnickle et al. 1991, Lamhamedi et al. 1998).

**Materials and methods**

**Plant material and growth conditions**

Seeds used to produce somatic and zygotic seedlings were from controlled crosses conducted among selected genotypes of white spruce by the Canadian Forest Service–Québec (Beaulieu 1996). After isolation of embryos from surface-sterilized seeds, embryogenic tissues were induced and maintained on HLM-1 medium according to Tremblay (1990) for a maximum of 6 months before being induced to mature. When embryogenic tissue that emerged from embryo explants had attained a size of about 3–5 mm in diameter, which occurred after 5–6 weeks of incubation, it was transferred to fresh induction medium for proliferation. For all clones, somatic embryos were matured in the presence of 45 µM ABA as described by Isabel et al. (1993). After 5 weeks in maturation medium, normal mature somatic embryos were germinated according to Khelifi and Tremblay (1995). Four families and 12 clones (three per family) were tested (Table 1).

In April 1997, plantlets with an epicotyl at least 1 cm long were transferred to Styroblock® (Beaver Plastics Ltd., Edmonton, Canada) cavities (45 cavities per block, 340 cm³ per cavity) filled with a moistened mixture of peat moss and vermiculite (3:1, v/v), and acclimatized in a greenhouse as previously described (Khelifi and Tremblay 1995). After 3 months in the greenhouse, survival rate was 96–100%. Seeds of the families corresponding to the clones were sown directly in the cavities the same day as the somatic plantlets were transferred to soil. A previous comparison among the same four families of white spruce germinated either in vitro or in vivo did not reveal any significant differences in either height or root-collar diameter after 7 months under greenhouse conditions. Somatic and zygotic seedlings were planted in a randomized complete block design, in which the multi-cavity Styroblocks® were the blocks and the randomization was performed between the cavities in each Styroblock®. Seedlings were grown for 6 months in a greenhouse at 23 ± 3 °C and 50–60% RH. Supplemental lighting from sodium vapor lamps was used to maintain a 19-h photoperiod. Mineral nutrients were

<table>
<thead>
<tr>
<th>Family or somatic seedlings</th>
<th>Clones or somatic seedlings</th>
<th>Height (cm)</th>
<th>Root-collar diameter (mm)</th>
<th>Needle dry mass (g)</th>
<th>Dry mass of branches (g)</th>
<th>Branch density (no. cm⁻¹)</th>
<th>Shoot dry mass (g)</th>
<th>Root dry mass (g)</th>
<th>Needle length (mm)</th>
</tr>
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<tbody>
<tr>
<td>C317</td>
<td>G304</td>
<td>15.1 ± 0.3</td>
<td>5.12 ± 0.29</td>
<td>2.67 ± 0.28</td>
<td>0.57 ± 0.11</td>
<td>1.03 ± 0.18</td>
<td>3.97 ± 0.33</td>
<td>2.08 ± 0.87</td>
<td>11.0 ± 0.5 675</td>
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<tr>
<td></td>
<td>G305</td>
<td>18.7 ± 0.4</td>
<td>5.26 ± 0.30</td>
<td>3.39 ± 0.28</td>
<td>0.72 ± 0.16</td>
<td>1.26 ± 0.21</td>
<td>4.35 ± 0.42</td>
<td>2.42 ± 0.85</td>
<td>13.0 ± 0.6 654</td>
</tr>
<tr>
<td></td>
<td>G306</td>
<td>17.4 ± 0.4</td>
<td>5.13 ± 0.29</td>
<td>2.74 ± 0.26</td>
<td>0.71 ± 0.15</td>
<td>1.04 ± 0.11</td>
<td>4.67 ± 0.42</td>
<td>2.42 ± 0.85</td>
<td>13.0 ± 0.6 654</td>
</tr>
<tr>
<td>C460</td>
<td>G430</td>
<td>15.8 ± 0.3</td>
<td>4.89 ± 0.91</td>
<td>2.84 ± 0.67</td>
<td>0.74 ± 0.25</td>
<td>1.43 ± 0.21</td>
<td>4.22 ± 1.09</td>
<td>1.42 ± 0.19</td>
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<tr>
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<td>G431</td>
<td>17.2 ± 0.3</td>
<td>5.32 ± 0.47</td>
<td>2.28 ± 0.59</td>
<td>0.44 ± 0.15</td>
<td>1.10 ± 0.21</td>
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<td>1.75 ± 0.89</td>
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<td></td>
<td>G432</td>
<td>21.3 ± 1.6*</td>
<td>6.48 ± 0.23*</td>
<td>3.93 ± 0.51*</td>
<td>0.82 ± 0.08*</td>
<td>1.01 ± 0.26</td>
<td>6.12 ± 0.77*</td>
<td>2.80 ± 0.89</td>
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<tr>
<td></td>
<td>G433</td>
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<td>6.12 ± 0.53*</td>
<td>3.01 ± 0.49</td>
<td>0.79 ± 0.14*</td>
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<td>0.55 ± 0.10</td>
<td>0.83 ± 0.14</td>
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<td>0.64 ± 0.15</td>
<td>0.69 ± 0.08</td>
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<td>3.06 ± 0.84*</td>
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<td>31.8 ± 4.9*</td>
<td>6.27 ± 0.44*</td>
<td>3.04 ± 0.66</td>
<td>0.48 ± 0.08</td>
<td>0.55 ± 0.13*</td>
<td>5.24 ± 1.06</td>
<td>3.30 ± 0.82*</td>
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<tr>
<td>C428</td>
<td>G435</td>
<td>22.0 ± 2.6</td>
<td>5.54 ± 0.76</td>
<td>2.82 ± 0.45</td>
<td>0.63 ± 0.11</td>
<td>1.05 ± 0.27</td>
<td>4.18 ± 0.49</td>
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<td></td>
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<td>20.3 ± 1.4</td>
<td>5.58 ± 0.83</td>
<td>3.22 ± 0.88</td>
<td>0.56 ± 0.23</td>
<td>0.90 ± 0.12</td>
<td>4.73 ± 1.24</td>
<td>1.98 ± 0.61</td>
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<tr>
<td></td>
<td>G439</td>
<td>21.0 ± 1.9</td>
<td>5.71 ± 0.83</td>
<td>2.76 ± 0.42</td>
<td>0.48 ± 0.19</td>
<td>0.99 ± 0.23</td>
<td>4.27 ± 0.54</td>
<td>2.16 ± 0.71</td>
<td>11.0 ± 0.9* 447</td>
</tr>
</tbody>
</table>

1 Number of needles used to estimate the mean length.

* Clones within a family that are significantly different from the zygotic seedlings (n = 8), using the Dunnett test (P = 0.05).
applied as mixed fertilizer solutions (20:8:20, 10:52:10 and 15:30:15 of N:P:K plus micronutrients; Plant Products Co. Ltd., Brampton, Canada) starting 2 weeks after transfer to the soil or seed sowing. During the 6-month growth period, each somatic or zygotic seedling received 41, 11 and 34 mg of N, P and K, respectively, plus micronutrients.

**Morphological variables and growth analysis**

At the end of the 6-month growth period, when seedlings had entered dormancy under greenhouse conditions, root-collar diameter, height, root and needle dry mass, number of first-order branches, shoot/root ratio and branch density (number of branches cm⁻¹ height) were measured on eight seedlings selected randomly from each clone and family tested. This sample size was found to be sufficient to estimate growth variables (Poorter and Garnier 1996). The dry mass of each component (needles, twigs and roots) was determined after drying at 65 °C for 48 h. Mean needle length was determined with WINNEEDLE software (Instruments Régent Inc., Québec, Canada). For each clone or family, the number of needles used to calculate mean needle length is indicated in Table 1.

**Tissue processing for microscopy**

Needle anatomy was investigated by light and electron microscopy on two families (C317 and C428) and four clones (G305, G306, G362, G369). For each family or clone, three to four needles were selected randomly from a composite sample (20–30 needles per family or clone). The middle portions (2-mm section) of needles were placed in a fixative solution consisting of 3% glutaraldehyde in 100 mM cacodylate buffer, pH 7.2. Samples were placed in a vacuum (51 kPa) for 1 h, then fixed overnight at 4 °C. They were then washed with the cacodylate buffer (3 × 20 min) and postfixed for 2 h at room temperature in 1% osmic acid (Karnovsky 1971) containing 2 mM potassium ferrocyanide and 6% sucrose in cacodylate buffer. After washing again with the buffer, samples were dehydrated in a graded series of ethanol and embedded in Epon (Polysciences, Halifax, Canada) according to Luft (1961). At least four samples were cut, and semi-thin sections were stained with 0.1% toluidine blue and examined with the aid of a Reichert-Jung Polyvar (Reichert-Jung Polyvar, Vienna, Austria) microscope. Photographs were taken with a technical PanSTAR (Kodak) film.

For transmission electron microscopy, ultrathin sections were deposited on nickel grids and stained with uranyl acetate and lead citrate. Observations were carried out with an electron microscope (JEOL, Model 1200X, Tokyo, Japan).

For scanning electron microscopy, sections were fastened to specimen holders with double-sided silver tape and fixed in osmic acid vapor for 48 h. They were then coated with gold using a sputter coater instrument. Observations were carried out with a scanning electron microscope (JEOL, Model JSM-35).

**Root system morphology and root growth potential (RGP)**

Unlike zygotic seedlings, somatic plantlets had to be transplanted from their in vitro germination medium to the styro-foam nursery containers. Transplanting of such delicate plantlets carries the risk of root misplacement in the new medium and subsequent root system deformation. Root morphology of the main taproot of eight somatic and eight zygotic seedlings was assessed as described by Grossnickle and Major (1994). Three categories were determined according to the deviation of the main taproot from a vertical position: (1) < 30°, (2) 30–90° and (3) > 90°.

Root growth potential (RGP) is the ability of a tree seedling to initiate and elongate roots when placed in an environment favorable for root growth (Simpson and Ritchie 1997). Eight seedlings per clone or family were transferred to 2-dm³ plastic pots filled with a moistened mixture of pot moss and vermiculite (3:1, v:v). Seedlings were watered with tap water to soil saturation and then grown for 21 days in a greenhouse at a mean day/night temperature of 25/18 °C, an 18-h photoperiod at a maximum photon flux density of photosynthetically active radiation (PPFD) of 800 µmol m⁻² s⁻¹, and a mean relative humidity of 50–60%. Seedlings showing bud set at the end of the growing period were used to assess RGP of each clone and family. New roots longer than 5 mm that grew out of the soil plug were counted and weighed after oven drying at 65 °C for 48 h.

**Mineral nutrition**

For dry matter determination, shoots were separated at the root collar and oven-dried at 65 °C for 48 h. After grinding and acid digestion, samples were subjected to total Kjeldahl nitrogen analysis and inductively coupled argon plasma analysis for P and K determination according to the method of Parkinson and Allen (1975). For each clone or family, three composite samples were used for nutrient analysis. Each composite sample was formed from two seedlings per clone or family.

Nitrogen-, phosphorus- and potassium-use efficiency (NUE, PUE and KUE) for each somatic or zygotic seedling was calculated as shoot biomass production/nutrient content (Ågren 1985) based on the mean mass of shoot seedlings pooled for nutrient analysis.

**Net photosynthesis (Pₙ)**

Instantaneous measurements of net photosynthesis (Pₙ) were made with a portable open-mode gas analyzer system with a cylindrical cuvette (Model LCA-4, Analytical Development Company, Hoddesdon, U.K.) on a lateral shoot of five seedlings per clone or family before evaluation of RGP. For measurements at photosynthetic photon flux density (PPFD) saturation, a sodium vapor lamp was suspended over the cuvette to maintain approximately 800 µmol m⁻² s⁻¹ at the top of each seedling, a value at which coniferous boreal species are usually light-saturated (Lamhamedi and Bernier 1994, Dang et al. 1997). The Pₙ was measured between 0900 and 1100 h only after seedlings had been illuminated for at least 3 h to ensure stomatal opening. Only lateral branches showing fully expanded needles were clamped into the chamber. Measurements of Pₙ were blocked across time to minimize confounding of time and clone or family effects. Each time block
consisted of one seedling from each of the 12 clones and four families. Immediately after measurements, needles inside the cuvette were removed for leaf area determination. The number of needles and their mean length were determined by image analysis with WINNEEDLE software (Instruments Régent Inc., Québec, Canada). To measure the perimeter of cross sections, three needles from each clone or family were fixed overnight at 4 °C in 4% formaldehyde in cacodylate buffer, then rinsed in buffer. Their perimeters were measured by image analysis of their middle cross sections. Perimeters and lengths were used to compute half of all-sided leaf area (hemisurface) (Chen et al. 1997). Net photosynthesis was expressed on a hemisurface basis.

Statistical analysis
All morphological and physiological data were analyzed as randomized complete block designs. When the results from analysis of variance were significant, a Dunnett test was used to determine if individual clones, within each family, were significantly different ($P = 0.05$) from the zygotic seedlings. Within each family, zygotic seedlings were considered as a control.

Variability in height, root-collar diameter, dry mass of new roots, needle dry mass, branch density, shoot dry mass, root dry mass, and needle length among clones within each family was compared with that observed for zygotic seedlings using a balanced model as described by Milliken and Johnson (1984). The statistical significance of all components of variance were determined at $\alpha < 0.3$ according to Milliken and Johnson (1984).

Results

Morphological variables and growth analysis
Under similar growth conditions, several clones differed significantly in various growth variables from the zygotic seedlings within the same family (Table 1, Figure 1). Differences were observed in height, root-collar diameter, needle dry mass, branch density, shoot dry mass, root dry mass, and needle length. Heights of clones and zygotic seedlings varied from 14.4 to 31.8 cm and from 15.8 to 24.3 cm, respectively. Root-collar diameter was generally greater in clones than in zygotic seedlings. Compared with the other clones and zygotic seedlings tested, plants from Clones G305, G318, G361 and G341 had noticeably higher needle and branch dry masses (Table 1). Differences in branch densities between clones and zygotic seedlings were significant in two (C460 and C567) of the four families. Within each clone and family (zygotic seedlings), shoot morphology and growth were similar across blocks, indicating strong genotypic control (Figure 1, Table 1). Among clones, the tallest, G368, had the longest needles and the lowest branch density. Mean needle length of the 12 clones ranged from 11 to 17 mm. Variation within families was larger between clones than between zygotic seedlings for height ($P =$
0.0015), needle dry mass \((P = 0.2776)\) and branch density \((P = 0.0871)\).

**Light microscopy**

Needles from zygotic and somatic seedlings had a similar tissue organization. Tannins, however, were more abundant in somatic than in zygotic seedlings. Furthermore, the distribution of tannins varied among genotypes. In Clone G305, tannins occurred as a ribbon along the central vacuole of mesophyll cells. This ribbon was frequently seen in association with bubble-like structures (Figure 2), and seemed to extend throughout the cytoplasm of numerous cells, conferring an overall dark staining to these cells (Figure 2). In Clones G306, G362 and G369, tannins occurred as numerous aggregates dispersed throughout the central vacuole of mesophyll cells (Figure 3). In the two zygotic seedlings observed (C317 and C428), tannins appeared as a thin ribbon associated with a bubble network (Figure 4). In some samples, however, a few aggregates were seen. Starch granules were also observed in all samples, with no marked difference between samples, except for Clone G305, where the starch granules were larger than in other samples.

**Electron microscopy**

Except for tannins, all needle samples displayed a similar ultrastructure, including the cuticular surface with its three-layered organization. Tannins exhibited a typical dark and striated appearance when occurring either as a ribbon surrounding the vacuole or as aggregates dispersed throughout the central vacuole (dark structures in Figures 5 and 6). The cytoplasmic dark staining observed by light microscopy (Figure 2) was a result of impregnation of the whole cytoplasm with tannins or other phenolic compounds. In many cases, mitochondria and chloroplasts were also impregnated with this dark material.

Scanning electron microscopy of the needle surface revealed that all needle samples displayed similar morphological features with almost amorphous or filamentous waxes that were often tube-like and distributed as tufts (Figures 7 and 8). In both clones and zygotic seedlings, stomata were covered by wax plugs that occluded the openings (Figures 7 and 8).

**Root-system morphology and root growth potential (RGP)**

The manual transplantation of somatic plantlets from test tubes to Styrobloc® containers resulted in numerous root deformations. Root systems of 52, 31 and 17% of all somatic seedlings were classified as Category 1 (taproot deviation < 30°), 2 (30–90°) and 3 (> 90°), respectively. Category 1 roots have a normal root form comparable with that observed in zygotic seedlings that were not transplanted (Figure 9a). However, severe malformations were occasionally observed such as asymmetrical roots, twisted primary roots, J-roots, swollen root-bases and knotted roots (Figures 9b–f). In asymmetrical roots (Figure 9b), the original taproot ceased to grow normally and lateral or replacement roots assumed the taproot function. The swelling just above the root collar resulted from root deformations. Root systems of 52, 31 and 17% of all somatic seedlings were classified as Category 1 (taproot deviation < 30°), 2 (30–90°) and 3 (> 90°), respectively. 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Category 1 roots have a normal root form comparable with that observed in zygotic seedlings that were not transplanted (Figure 9a). However, severe malformations were occasionally observed such as asymmetrical roots, twisted primary roots, J-roots, swollen root-bases and knotted roots (Figures 9b–f). In asymmetrical roots (Figure 9b), the original taproot ceased to grow normally and lateral or replacement roots assumed the taproot function. The swelling just above the root collar resulted from root
strangulation and confirmed the persistence of root deformation during the growing period.

Root growth potential showed significant clonal differences in both the number and dry mass of new roots within a family (Figure 10). Compared with zygotic seedlings from the corresponding families, some clones (G318, G357 and G368) showed a higher rooting ability in terms of the number or dry mass of new roots, whereas other clones (G304 and G362) showed a lower rooting ability (Figure 10a–h). The number of new roots ranged from 200 to 270 and from 90 to 130 new roots for good- and poor-rooting clones, respectively. Dry mass of new roots varied from 0.38 to 0.68 g and 0.20 to 0.30 g for good- and poor-rooting clones, respectively. In contrast, there was no difference in either number or dry mass of new roots between Clones G305, G306, G361, G341, G342, G355 and G369 and the zygotic seedlings from the corresponding families. The variation in dry mass of new roots was larger among clones than among zygotic seedlings (P = 0.0322). Uniformity in the ability of each clone to initiate new roots confirmed that the differences in rooting ability were under genetic control.

Mineral nutrition

Within families, clonal variations were observed in N, P and K use efficiencies (g shoot dry mass/g of element) (Table 2). For all three elements, large differences were observed between the clones and zygotic seedlings of family C460. The fast-growing Clones G318, G341 and G342 had significantly higher nutrient-use efficiencies for N, P and K than the corresponding zygotic seedlings. In contrast, some fast-growing clones, for example G305, did not differ significantly from the zygotic seedlings from the same family in nutrient-use efficiency for N, P or K. Only Clone G369 showed lower N-
P-use efficiencies than the zygotic seedlings from its family. In these well-fertilized seedlings, nutrient-use efficiency was not well related to biomass growth.

Net photosynthesis ($P_n$)

In general, clones had higher $P_n$ than zygotic seedlings from the same family (Figure 11), but the difference was significant only for Clones G318, G357, and G368. The fast-growing Clone G368 had a higher $P_n$ than that of most other clones and all of the zygotic seedlings in its family.

Discussion

Within all four families, there were significant differences between zygotic seedlings and clones for several growth and physiological variables (Figures 1, 10 and 11; Tables 1 and 2). Natural variability exists within families and differences among clones were expected, because each clone was grown from a single seed. However, we found greater variability in height ($P = 0.0015$), dry mass of new roots ($P = 0.0322$), needle dry mass ($P = 0.2776$) and branch density ($P = 0.0871$) among clones than among zygotic seedlings within families. This finding is in agreement with the observations of Fries and Kaya (1997), and indicates that some growth traits can be improved by clonal selection. Intraclonal uniformity as well as the uniformity of greenhouse growth conditions for all clones and zygotic seedlings suggest that differences among clones were of genetic origin. Studies with *Picea abies* (L.) Karst. have demonstrated differences between clones within families for initiation, proliferation and cryopreservation of embryogenic cultures, maturation of somatic embryos, and plant regeneration (Högberg et al. 1998). In addition to genetic effects, clonal variability could also be associated with the selection pressure exerted on the seed population during the induction step, with the selection of the most productive embryogenic lines for plant production, or with an unknown mechanism.

Little is known about genetic variability among coniferous clones at the seedling stage, but for several tree species, morphological and physiological characteristics such as those used in this study have been correlated to field growth characteristics expressed after several growing seasons (Ying and Morgenstern 1979, Nienstaedt 1981, Ceulemans et al. 1987, Li et al. 1993, Sulzer et al. 1993), indicating that early selection of superior clones based on both physiological and morphological characteristics is feasible.

Recent studies have begun to shed light on clonal differences in growth and physiological variables of coniferous somatic seedlings. Prior to field planting, Grossnickle et al. (1996) reported a large variation in height and root-collar diameter between clones of interior spruce (*Picea glauca × Picea engelmannii*). On the other hand, it was shown that the means of physiological and morphological variables measured on zygotic and somatic seedlings from pooled genotypes were mostly similar during two consecutive growing seasons on a reforestation site (Grossnickle and Major 1994).

We found significant differences in $P_n$ among clones within the same family (Figure 11) that were correlated with biomass of new roots and NUE (results not shown), suggesting the importance of early identification of clones with high $P_n$. Other studies (Major and Johnsen 1996, Sun et al. 1996, Fan and Grossnickle 1998) have also demonstrated the usefulness of $P_n$ in genetic selection. Thus, for interior spruce clones grown under simulated drought conditions, $P_n$ was more sensitive than the zygotic seedlings from its family.
shoot water relations parameters in detecting interclonal variations (Fan and Grossnickle 1998). Grossnickle and Fan (1998) also detected interclonal variation in gas exchange variables under optimum edaphic conditions, but over a wide range in light and vapor pressure deficit conditions. Environmental conditions in vitro did not affect the subsequent development of cuticle, epicuticular and epistomatal wax features (Figures 7 and 8), indicating that the variations in $P_n$ among clones are under genetic control. These waxes are known to be important in the maintenance of plant water balance (Jeffree et al. 1971).

Differences in growth variables (Table 1) and nutrient status (Table 2) among clones can be a result of differences in both uptake and physiological use efficiency of nutrients and photosynthetic products. Clonal variability was also observed in other macro- and micronutrients (data not shown). For example, in spite of fertilizer application, plants from specific

Figure 10. Comparison of means for the number (a–d) and dry weight (e–h) of new roots between zygotic seedlings (C317, C460, C567 and C428) and clones of white spruce. Asterisks indicate clones, within individual families, that are significantly different ($P \leq 0.05$) from the zygotic seedlings according to the Dunnett test.
clones suffered from typical copper deficiency symptoms in all blocks both in this study and in other trials during their exponential growth phase.

The partitioning of newly synthesized photosynthates between different plant parts is also likely to vary clonally (Cannell et al. 1983). Developmental and growth partitioning differences have been observed among several clones and families of white spruce during initiation, maturation and germination of embryogenic tissue (Park et al. 1993, 1994). The rapid development of the shoot and root system and the improvement of shoot architecture that we observed in several clones indicates that the potential exists to select for improved productivity of white spruce plantations. Studies with Picea abies have shown that clonal selection in the nursery gave

Table 2. Comparison of means of nutrient-use efficiencies for nitrogen (NUE), phosphorus (PUE) and potassium (KUE) between zygotic and somatic seedlings of Picea glauca within each family.

<table>
<thead>
<tr>
<th>Family or zygotic seedlings</th>
<th>Clones or somatic seedlings</th>
<th>NUE</th>
<th>PUE</th>
<th>KUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C317</td>
<td>G304</td>
<td>55.97±2.49</td>
<td>297.11±5.58</td>
<td>131.22±7.17</td>
</tr>
<tr>
<td></td>
<td>G305</td>
<td>58.91±1.79</td>
<td>362.84±22.53</td>
<td>148.69±9.20</td>
</tr>
<tr>
<td></td>
<td>G306</td>
<td>54.48±2.06</td>
<td>346.36±2.01</td>
<td>145.86±6.48</td>
</tr>
<tr>
<td>C460</td>
<td>G318</td>
<td>42.64±2.08</td>
<td>327.26±16.29</td>
<td>123.87±9.49</td>
</tr>
<tr>
<td></td>
<td>G357</td>
<td>54.09±2.93*</td>
<td>401.74±4.25*</td>
<td>141.30±8.72</td>
</tr>
<tr>
<td></td>
<td>G361</td>
<td>55.47±1.41*</td>
<td>354.95±0.59</td>
<td>120.03±3.99</td>
</tr>
<tr>
<td>C567</td>
<td>G341</td>
<td>48.15±1.61</td>
<td>294.19±10.56</td>
<td>141.29±4.33</td>
</tr>
<tr>
<td></td>
<td>G342</td>
<td>56.22±1.04*</td>
<td>408.29±11.32*</td>
<td>205.89±7.88*</td>
</tr>
<tr>
<td></td>
<td>G341</td>
<td>55.94±0.85*</td>
<td>378.66±0.88*</td>
<td>186.69±4.56*</td>
</tr>
<tr>
<td>C428</td>
<td>G355</td>
<td>50.91±2.34</td>
<td>309.51±2.82</td>
<td>163.46±2.24</td>
</tr>
<tr>
<td></td>
<td>G356</td>
<td>54.08±1.73</td>
<td>332.55±12.05</td>
<td>123.33±6.30</td>
</tr>
<tr>
<td></td>
<td>G356</td>
<td>46.22±2.91</td>
<td>293.16±7.18</td>
<td>125.10±6.88</td>
</tr>
<tr>
<td></td>
<td>G369</td>
<td>60.01±3.12</td>
<td>399.18±6.41*</td>
<td>140.64±5.02</td>
</tr>
<tr>
<td></td>
<td>G369</td>
<td>41.36±1.62*</td>
<td>230.89±7.43*</td>
<td>123.32±7.11</td>
</tr>
</tbody>
</table>

* Clones within the same family that are significantly different from the zygotic seedlings (n = 3), using the Dunnett test (P = 0.05).

Figure 11. Comparison of mean net photosynthetic rates ($P_n$) between zygotic seedlings (C317, C460, C567 and C428) and clones of white spruce. Asterisks indicate clones, within individual families, that are significantly different ($P \leq 0.05$) from the zygotic seedlings according to the Dunnett test.
gains of 2.4 to 3.4% in height after 6 years under different site conditions (Högberg and Karlsson 1998). Boltz et al. (1986) observed that loblolly pine seedlings from a fast-growing provenance had higher rates of net photosynthesis than slow-growing provenances. Paterson and Fayle (1984) showed that root establishment and survival in the first 2 years after planting of Pinus resinosa Ait. seedlings were positively correlated to the length of needles. Compared with a slow-growing clone, a fast-growing clone like G368, with high net photosynthesis, long needles, low branch density and high dry mass of new roots, could compete more efficiently for light, water and nutrients on sites with dense competing vegetation and thereby reduce the cost of plantation establishment. In the province of Québec, Canada, large seedlings of several boreal species are used in reforestation programs as a possible alternative to the use of herbicides (Lamhamedi et al. 1998). Clones showing high nutrient-use efficiency, such as G318, could be used for reforestation of sites with low soil fertility.

After one growing season, tannins were more abundant in needles of clones than in needles of zygotic seedlings (Figures 5 and 6). Depending on the genotype, tannins were distributed as a ribbon or as aggregates dispersed throughout the central vacuole. In Norway spruce needles, the relative proportions of these two forms of tannins were related to seasonal changes (Soikkeli 1978). In our study, differences in tannin distribution were attributed to genotypic effects rather than to seasonal changes. Populus tremuloides Michx. also exhibits substantial interclonal variation in leaf chemistry, including concentrations of tannins and phenolic glycosides (Lindroth et al. 1996, Hwang and Lindroth 1997, Mansfield et al. 1999). Similar variations in leaf chemistry have been reported in hybrid and parental willows (Orians and Fritz 1995), suggesting that genetic factors are responsible for this interclonal variation. Hwang and Lindroth (1997) reported that clonal variation in tannin production contributed to differential performance of insects feeding on aspen clones. Differences among clones (G305 versus G306, G362 and G369) in the distribution of tannins have important implications for pest management. Clones with high concentrations of tannins distributed in the ribbon form should efficiently resist fungal or insect attacks. Tannins precipitate proteins, inhibit most enzyme reactions, and make the proteins present in plant tissue nutritionally unavailable to most insects and fungi (Zucker 1983). Quiring et al. (1991) showed the presence of genetic variations among families of Picea glauca in their susceptibility to the spruce bud moth. Characterization of resistance mechanisms of white spruce may prove useful in identifying susceptible or resistant clones in genetic improvement and reforestation programs.

Rooting capacity can also be improved by clonal selection. Several clones had both high RGP and high stem elongation rate (Figures 1 and 10), indicating that selection of white spruce clones based on RGP would not exclude clones with high aboveground growth. Variations in rooting ability among white spruce clones produced through somatic embryogenesis are consistent with findings for cuttings of other tree species (Bentzer 1988, Kormanik et al. 1990, Pounders and Foster 1992, Fries and Kaya 1997). There is also substantial evidence that there is genetic variation in rooting ability and nutrient uptake among families (Theodorou and Bowen 1993). The development of new roots is closely dependent on the availability of current photosynthates (van den Driessche 1987, Kozlowski 1992), particularly starch (Rose 1992). Clones with high RGP generally showed high Pn and such clones can re-establish soil-root contact more rapidly than poor-rooting clones following transplanting. Clonal differences in RGP may also have considerable effects on the uptake of the poorly mobile ions from soil.

Transplanting somatic plantlets can result in root deformations (cf. Grossnickle and Major 1994). Hay and Woods (1975) showed that these deformities induced accumulation of carbohydrates close to the root collar, which increased the development of lateral roots. Although root deformities of the Category 2 type do not greatly influence root development in potted seedlings (Scarratt 1991) or in the field (Girouard 1992), root deformations of the Category 3 type can affect subsequent growth (Grene 1978). However, root deformation can be eliminated by proper handling or automation of somatic plantlet transplantation (Roberts et al. 1995). Picea species can also sometimes overcome root deformation by forming adventitious roots after plantation. In the boreal forest ecosystems, it is known that lateral roots are critical for water and nutrient uptake, whereas deeper roots are critical for stability and play an important role during episodic drought periods (Lamhamedi and Bernier 1994).

We conclude that clones of white spruce regenerated from somatic embryos are suitable for reforestation programs. Many clones also show faster height growth, longer needles, and different tannin structure and distribution compared with the parent family. We postulate that the somatic embryogenesis (SE) pathway will increase our capacity to select genotypes with desired traits such as rapid growth, wood quality and tolerance to environmental stresses; however, this hypothesis will have to be validated with older clonal plantations produced through SE. We are now investigating the adaptability of these clones over a range of sites across the province of Québec. This information will enable us to match clones having specific characteristics to specific sites and provide a basis for selecting clones that perform moderately well in a broad range of environments.

Acknowledgments

We thank Dr. Jean Beaulieu, from the Canadian Forest Service, for providing the seeds from controlled crosses. We also thank Caroline Levasseur and Laurence Tremblay for helpful discussions and for the production of all seedlings, Martine Blais and Martine Moreau for editorial comments. Thanks are also extended to Dr. Hank Margolis for his critical review of the manuscript. This research program was supported by a grant to FMT from the ministère de l’Industrie et du commerce, science et technologie (programme Synergie), in partnership with CPPFQ Enr., PAMPEV INC., and BECHEDOR Inc.
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