Influence of environment, fertilizer and genotype on shoot morphology and subsequent rooting of birch cuttings

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Summary  Differences in rooting ability of birch (Betula pubescens J.F. Ehrh.) cuttings were observed as a result of differences in genotype and physiology of the stock plants. The uniformity in response among cuttings from micropropagated plants compared with cuttings from seed plants confirmed the advantage of using micropropagated plants to study environmental effects. Shoot morphology of the seed stock plants was influenced by both photoperiod and thermoperiod. A day/night temperature of 15/25 °C reduced stem elongation compared with a day/night temperature of 25/15 °C regardless of photoperiod, and a continuous light regime resulted in more shoots per plant in both temperature regimes than a 16-h photoperiod. A reduction in the supply of macronutrients did not influence rooting percentage but increased rooting substantially and seemed to override the effects of environmental factors. In cuttings of seed plants, the highest rooting percentage and number of roots were obtained in a 16-h photoperiod with a day/night temperature of 15/25 °C. Root branching increased with temperature. At all temperatures, there was a positive correlation between shoot length and number of leaves per shoot and topographical distribution of light within the plants, but there was no correlation between these parameters and rooting ability of the cuttings. A rooting temperature of 16 °C delayed the rate of root production compared with the rate at higher temperatures, but the final rooting percentage was the same over the range from 16 to 28 °C. Root branching increased with temperature. At all temperatures, there was a large increase in sucrose content at the base of the cuttings during rooting, whereas the concentration of nontranslocated sugars remained constant. The carbohydrate content at the base of cuttings from micropropagated stock plants was three times higher than at the base of cuttings from seed stock plants, but the higher carbohydrate content was not correlated with a higher rooting potential.

Keywords: Betula pubescens, hydroponic system, micropropagation, photoperiod, temperature, thermoperiod.

Introduction

Seasonal variations in rooting ability of cuttings emphasize the importance of identifying the physiological status of the stock plant when cuttings are taken (Nanda and Anand 1970, Anand and Heberlein 1975, Hansen and Ernstsen 1982). The opportunity to manipulate the rooting potential of cuttings is generally much greater when favorable conditions are given to the stock plants rather than the cuttings taken from them (Howard and Harrison-Murray 1988, Howard 1991). The mineral nutrient status of the stock plant affects rooting; a low N supply improves rooting (Haun and Cornell 1951, Preston et al. 1953).

Photoperiod (Bachelard and Stove 1963, Heide 1965, Nanda et al. 1967) and irradiance (Fisher and Hansen 1977, Poulsen and Andersen 1980) also influence the rooting ability of many plants. Although irradiance has not always been associated with high rooting potential (Okoro and Grace 1976, Hansen et al. 1978, Veierskov and Andersen 1982, Veierskov et al. 1982a), it has been used to change the carbohydrate content of cuttings. The need for carbohydrates during root initiation was first demonstrated by Borthwick et al. (1937) and has been verified by Bhattacharya et al. (1976). However, the amount of carbohydrate required in this process is not known, and the relationship between carbohydrates and adventitious root formation is unclear (Haissig 1984, Veierskov 1988).

The optimal root temperature for rooting cuttings is difficult to determine, because the effects of temperature are often associated with other features of the propagation environment and with genotype (Andersen 1986).

There is substantial evidence that rooting ability is genetically controlled (Haissig and Riemenschneider 1988). Clonal differences in rooting ability of cuttings from several forest tree species have been reported (Dunn and Townsend 1954, Ying and Bagley 1977, Pounders and Foster 1992). However, it is important in genetic studies of rooting that confounding environmental effects are separated and properly controlled. One way to separate the nongenetic effects of the stock plant from the genetic effects is to compare cuttings from seed stock plants with cuttings from micropropagated stock plants grown under controlled conditions.

I have studied the effects of photoperiod, thermoperiod and fertilizer on shoot morphology and subsequent rooting of birch cuttings, and genotypic differences in rooting ability by comparing seed plants with micropropagated plants. I also examined whether the topographical distribution of light within the plants was related to shoot morphology and subsequent rooting. The influence of temperature during rooting in a hydroponic system was examined and attempts were made to...
correlate rooting capacity of cuttings from cloned material and seed plants to the amount of carbohydrate in the stem base of the cuttings during rooting.

Material and methods

Seed plant cuttings

Seed collection and growing conditions. In 1987, seeds were collected from an early flowering genotype of *Betula pubescens* J.F. Ehrh., growing at the University College of Wales, and brought to Sweden. The seeds were sown in a greenhouse and the seedlings were planted in a field in July 1988. Seeds from these trees were harvested in September 1989, sown in the greenhouse on October 16, 1989, and then planted in pots containing Hasselfors Master Soil 14 days later. The seedlings were grown in the greenhouse in natural daylength at 18 °C until February 2, 1990. The plants, which were then approximately 30 cm tall, were selected to serve as seed stock plants based on uniformity and placed in four climate chambers under the conditions described in Table 1. Irradiance, measured at plant level, was supplied by a combination of incandescent and cool-white fluorescent lamps. Each climate chamber contained approximately 30 cm tall, were selected to serve as seed stock plants based on uniformity and placed in four climate chambers under the conditions described in Table 1. Irradiance, measured at plant level, was supplied by a combination of incandescent and cool-white fluorescent lamps. Each climate chamber contained 28 seed stock plants and half of the plants were watered with Nutrient Solution I (macronutrients (g l⁻¹) K₂PO₄ 13.6, KNO₃ 20.6, Ca(NO₃)₂ ⋅ 4H₂O 23.6, Fe- Na-EDTA 7.3, MgSO₄ ⋅ 7H₂O 25.0 (diluted 1/100); micronutrients (mg l⁻¹) MnSO₄ ⋅ 4H₂O 89.0, CuSO₄ ⋅ 5H₂O 12.0, Na₂MoO₄ ⋅ 2H₂O 0.2, ZnCl₂ 6.0, H₂BO₃ 24.7 (diluted 1/1000)) and the other half with Nutrient Solution II (identical to Nutrient Solution I except that macronutrient concentrations were diluted 1/200). Time of transfer to the climate chambers is referred to as Day 0 in the figures.

Plant height, number of shoots per seed stock plant and the irradiance at each shoot used as a cutting were recorded at about 7-day intervals for 25 days. At the end of the experiment the total irradiance that each shoot had received during the growth period was calculated, and shoot length and number of leaves per shoot were recorded. In April 1990, four seed stock plants approximately 100 cm in height were randomly selected from each climate chamber for rooting experiments.

Rooting experiments with cuttings from seed plants. Five shoots per seed stock plant (numbered 1–5 counted from the base) were used as cuttings. The cuttings were trimmed to a final length of 15 cm and placed in reservoirs containing Nutrient Solution II. Air was bubbled through the reservoirs. Four reservoirs with temperatures of 16, 20, 24 and 28 °C were used with 20 cuttings per temperature treatment. The reservoirs were placed in climate chambers providing a 16-h photoperiod and a relative air humidity (RH) of 90%. The reservoirs were covered with plastic, white on top and black underneath to minimize algae formation. The rooting percentage and number of roots were recorded at about 3-day intervals for 25 days.

Micropropagated plant cuttings

Growing conditions, tissue culture and rooting. Seeds were sown in the greenhouse on September 28, 1990, and the seedlings were planted in pots containing Hasselfors Master Soil on October 15 and grown in the greenhouse in a 16-h photoperiod (160 µmol m⁻² s⁻¹) with a day/night temperature of 15/25 °C and 70% RH (Climate B16 in Table 1). The plants were watered with Nutrient Solution II.

Micropropagated stock plants were produced according to the method described by Welander (1988). On August 23, the micropropagated stock plants were transferred to a 1/1 (v/v) mixture of peat and perlite, covered with plastic beads and placed in the greenhouse. After one week, the beads were replaced by a plastic tent and the plants were hardened off by removing the tent for successively longer periods each day.

On September 18, the micropropagated stock plants were transferred to pots containing Hasselfors Master Soil and grown in the greenhouse in a 16-h photoperiod (160 µmol m⁻² s⁻¹) with a day/night temperature of 15/25 °C and 70% RH (Climate B16 in Table 1) until November 1. Forty micropropagated stock plants were then placed in each of four climate chambers under the conditions described in Table 1.

Plant height, number of shoots and the irradiance at each shoot used as a cutting were recorded at about 7-day intervals. At the end of the experiment, the total irradiance each shoot had received during the growth period was calculated, and shoot length and number of leaves per shoot were recorded.

Rooting of cuttings from micropropagated plants. Six shoots (numbered 1, 3 and 5 on one side, and 2, 4 and 6 on the opposite side counted from the base) from each micropropagated stock plant were used as cuttings. The cuttings were placed in reservoirs as described for cuttings from seed stock plants. Four reservoirs with temperatures of 16, 20, 24 and 28 °C were used with 20 cuttings per temperature treatment. The rooting per-

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Table 1. Description of the four climate treatments to which the stock plants were exposed for two months. The relative humidity was 70% and the irradiance was 160 µmol m⁻² s⁻¹ in all of the treatments.

<table>
<thead>
<tr>
<th>Climate</th>
<th>Photoperiod (h)</th>
<th>Thermostream (h)</th>
<th>Day/night temperature (°C)</th>
<th>Irradiance (µmol m⁻² day⁻¹)</th>
<th>Average day temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A24</td>
<td>24</td>
<td>16/8</td>
<td>25/15</td>
<td>3840</td>
<td>21.7</td>
</tr>
<tr>
<td>B24</td>
<td>24</td>
<td>16/8</td>
<td>15/25</td>
<td>3840</td>
<td>18.3</td>
</tr>
<tr>
<td>A16</td>
<td>16</td>
<td>16/8</td>
<td>25/15</td>
<td>2560</td>
<td>25</td>
</tr>
<tr>
<td>B16</td>
<td>16</td>
<td>16/8</td>
<td>15/25</td>
<td>2560</td>
<td>15</td>
</tr>
</tbody>
</table>
The percentage and number of roots were recorded at about 3-day intervals for 25 days.

Measurement of carbohydrates
During rooting, the basal 1.0 cm of the stem from three shoots per plant was analyzed for glucose, fructose and sucrose. Samples (30–50 mg FW) were taken at Days 0, 7, 10 and 14. The samples were freeze dried and extracted in buffered methanol (0.1 M acetate buffer, pH 6.6, containing methanol, 2/8 v/v) in a water bath at 55 °C for 30 min. The extracts were shaken several times during the day and clarified overnight. The clear supernatant was analyzed enzymatically for glucose, fructose and sucrose (Boehringer Mannheim, Mannheim, Germany).

Statistical analyses
Data in Figure 1 were subjected to analysis of variance with SAS software (SAS Institute Inc., Cary, NC, USA) and Tukey’s studentized range test for multiple comparison of means. In Figure 3, significance was determined by Fisher’s exact test for percent rooting and Welch’s approximate t-test in pairs (Zar 1984) for root number. Linear regression analysis was used for calculations of relationships between shoot length, number of leaves per shoot and irradiance.

Results
Shoot morphology of stock plants
Figure 1 shows the average seed plant height (A) and the average number of shoots per seed plant (B) in four different climates including two nutrient regimes. Seed plant height in Climate A24 was significantly higher than in Climate B24, and seed plants in Climate A16 were significantly taller than in Climate B16, indicating that temperature had a greater influence on seed plant height growth than daylength. The shortest, most compact seed plants were in Climate B16 and the tallest seed plants with the longest internodes and longest shoots were in Climate A24 (Figure 2). Significantly more shoots were produced in Climates A24 and B24 than in Climates A16 and B16, indicating that number of shoots per seed plant was influenced more by daylength than temperature. The nutrient treatments did not affect either seed plant height or the number of shoots.

Micropropagated stock plants were more uniform in height (Figure 3) and number of shoots per plant (Figure 4) than plants derived from seeds. In micropropagated plants, there was a positive relationship between final axillary shoot length and light intensity \( r = 0.633 \) and between number of leaves per shoot and light intensity \( r = 0.631 \). In seed plants, the
correlation coefficients for final shoot length and light intensity ($r = 0.470$) and for number of leaves and light intensity ($r = 0.367$) were lower.

Rooting of cuttings

The rooting ability of cuttings taken from seed stock plants subjected to the different climates is shown in Figure 5. Variation in rooting ability within treatments was high, probably because of genotypic differences in rooting capacity between individual seed stock plants, and only a few treatments differed significantly. Rooting percentage and the number of roots was analyzed by Fisher's exact test and root number was analyzed by Welch's approximate $t$-test in pairs.
increased by Nutrient Solution II compared with Nutrient Solution I, and the continuous light regime inhibited rooting of cuttings compared with the 16-h photoperiod. The highest rooting percentage and number of roots per cutting were obtained from seed stock plants grown in Climate B16 with Nutrient Solution II. This stock plant treatment was chosen for pretreating the micropropagated stock plants.

Shoot length and number of leaves per shoot were unrelated to percent rooting or number of roots per cutting. Both root branching and root length increased with increasing rooting temperature (Figure 6).

Genotypic differences in the rooting ability of cuttings from seed plants were observed at all rooting temperatures. The genotypic variations dominated the temperature effects on rooting percentage (Figure 7A) and number of roots per rooted cutting (Figure 7B). A temperature of 16°C increased the time required for rooting (Figure 8A), but the final rooting percentage and number of roots (Figure 8B) were the same or slightly higher compared to those in the other temperature treatments. No differences in rooting were observed at rooting temperatures of 20, 24 and 28 °C. Cuttings from micropropagated plants achieved optimum rooting percentage one week earlier than cuttings from seed plants at all temperatures except 16°C.

**Carbohydrate content and rooting**

The analysis of fructose, glucose and sucrose in the basal 1.0 cm of the stem during rooting showed a similar pattern in cuttings from both micropropagated trees and seed plants (Figures 9 and 10). The fructose and glucose contents were constant over the rooting period, whereas there was a large accumulation of sucrose between Days 7 and 10 in all of the temperature treatments. The endogenous carbohydrate content
was almost three times higher in the stem base of the cuttings from micropropagated plants than from seed plants (cf. Figures 9 and 10). However, differences in rooting percentage and number of roots were not correlated with carbohydrate content.

Discussion

Differences in rooting ability were the result of both genotypic differences and physiology of the stock plants. In this study, the difficulty of separating environmental effects from genetic differences was overcome by comparing cuttings from seed plants with cuttings from micropropagated cuttings (Burdon and Shelbourne 1973). The large variation in rooting capacity among the seed plants (Figure 6) exemplifies the potential for selecting clones with improved rooting characteristics (Fancher and Tauer 1981, Foster et al. 1984).

Plant height was influenced more by the temperature treatments than by the photoperiodic regimes. A higher night than day temperature reduced plant height, whereas a lower night
than day temperature stimulated stem elongation (cf. Moe and Heins 1990). Lateral branching is also affected by daylength (Healy et al. 1980). Although a reduced concentration of macronutrients did not limit plant height and number of shoots produced per stock plant, it increased the rooting ability of the cuttings significantly. Because gibberelins inhibit rooting in a variety of species and gibberellin content is decreased in plants subjected to a higher night than day temperature (Erwin et al. 1989), I hypothesized that cuttings from stock plants grown in a higher night than day temperature would root better than cuttings grown in a lower night than day temperature. The highest rooting percentage and number of roots was found in cuttings taken from stock plants grown in climate B16; however, a reduction in macronutrient supply dominated the effect of the environment. Stock plants given a reduced supply of macronutrients showed a higher rooting capacity under all climatic conditions. Pearse (1943) found that cuttings from plants grown under conditions of mineral starvation rooted easily, and Hyndman et al. (1982) showed that rooting was enhanced by lowering the salt concentration, which was mainly attributed to a lower nitrogen concentration.

The effects of temperature on rooting ability of cuttings from seed plants were difficult to interpret because of genotypic differences. In contrast, the results obtained with cloned material were unequivocal and showed that rooting was delayed at 16 °C compared with rooting at higher temperatures, but the final rooting percentage and number of roots were similar within the temperature range of 16 to 28 °C. However, root branching was greater at 20–28 °C than at 16 °C. Moore et al. (1975) reported an increase in the time required for rooting at low temperatures, and Takahashi et al. (1981) found that the final number of roots is higher at higher temperatures, which might be a consequence of a longer period available for root initiation.

Cuttings generally accumulate solubile carbohydrates at their bases during the early stages of root formation (Middleton et al. 1980, Veierskov et al. 1982*). The rapid accumulation of sucrose indicates that rooting is an energy demanding process but the amount of carbohydrate required for this process is not known. I observed a large accumulation of sucrose at the base of the cutting before any roots were visible. In cuttings of *Pinus banksiana* Lamb. (Haissig 1984) and pea (*Pisum sativum* L.) (Veirskov 1988), rapid accumulation of carbohydrates was observed two days after propagation. Jarvis (1986) suggested that adventitious rooting is dependent on sugar transported from the stele and not on sugar from the surrounding tissues. This suggestion is supported by the finding that there were only minor changes in nontranslocatable reducing sugars in birch cuttings in this study. The carbohydrate content of cuttings from micropropagated plants was about three times higher than that of cuttings from seed plants; however, the higher carbohydrate content was not correlated with a higher root initiation. Based on this observation and the finding that the increase in carbohydrate content was similar at all temperatures although rooting was delayed in the low temperature treatment, I conclude that carbohydrates do not control root initiation in birch cuttings. The large accumulation at the base of the cuttings indicates the need for an adequate supply of carbohydrates during rooting. Thus, sugar availability can limit adventitious rooting in leafless or defoliated cuttings (Fabijan et al. 1981) or if carbohydrates are depleted in the stock plant (Veierskov et al. 1976).

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**References**


