Seed Production of Micropropagated Plants, Grafts and Seedlings of Birch in a Seed Orchard

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Seed production of micropropagated plants, seedlings and grafts of silver birch (*Betula pendula* Roth) in a polythene greenhouse experiment was followed for five years. The grafts started flowering and seed production at the age of two years, one year earlier than other two types of material. At the age of three the seed production of both the micropropagated plants and seedlings was already more than two times higher than that of the grafts. Variation between the clones was high and plant type × clone interaction was significant. At the age of four, in 1993, seed production was high in all three types of material. Seed production of the micropropagated plants was two times higher than that of the grafts but about 75 % of that of the seedlings. In 1994 seed production of all three plant types was very low, which shows large variation between different years. The early development of the plant material types suggests that micropropagated birch plants have higher seed production than grafts and could well be used instead of grafts in polythene greenhouse seed orchards.

Keywords *Betula pendula*, tissue culture, grafting, seedlings, seed orchards, seed production.
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1 Introduction

The cultivation of birch has increased considerably in Finland in recent years, since both silver birch (*Betula pendula* Roth) and pubescent birch (*Betula pubescens* Ehrh.) are important raw materials for mechanical and chemical forest industry. Birch is now planted on nearly 20 % of the annual area of artificial regeneration (Aarne 1993). The birch seed used for seedling production has been obtained from polythene greenhouse seed orchards or collected from seed production stands.

At present the seed production capacity of existing seed orchards covers almost all the demand for nursery birch seed (Hagqvist 1991).

Using polythene greenhouse seed orchards, the mass production of genetically improved seed can be arranged at low cost and minimum space consumption (Lepistö 1973). Since the 1970's this method has become a well-established practice in the production of genetically improved seed in Finland (Foundation for Forest...1979, Hagqvist 1991). Most of the birch seed orchards are multi-clonal orchards. Bi-clonal seed orchards have also been established in order to produce full-sib families for practical cultivation (Hagqvist 1991).

A seed orchard can be established with the grafts of tested plus trees or seedlings selected from the best progenies. The weaker growth, branching habit and seed production of grafts compared to seedlings has sometimes been a problem (Tyystjärvi and Pirttilä 1984). On the other hand, seedlings cannot be used in bi-clonal seed orchards, because seedlings do not have genotypes identical to that of the parent trees. If specific parental combinations are detected by means of progeny testing, reproduction of the same combination is, of course, to be done with the original genotypes.

Cloning by micropropagation via tissue culture has several advantages compared to conventional methods of clonal propagation. There is, however, very little information on, for example, seed production of micropropagated birch plants.

In this study the seed production of micropropagated plants, grafts and seedlings of birch was followed to the age of five years in a polythene greenhouse experiment. The aim was to determine whether micropropagated plants can be used instead of grafts or seedlings in birch seed orchards designed for the mass production of genetically improved seed.

2 Material and Methods

The material consisted of seedlings, grafts and micropropagated plants originating from the same trees. The material was collected from a silver birch stand at Punkaharju, south-eastern Finland (61°49'N, 29°18'E, 90 m asl), planted with seedlings of local origin in 1932. Twenty twigs were collected from 10 random trees in the stand for tissue culture in winter 1988 and again for grafting in early spring 1989. Open-pollinated seed from the same trees had been collected in 1985.

Propagation by tissue culture started at Punkaharju research station in 1988, where potting of micropropagated plants, after 13–16 passages, grafting and sowing were done in spring 1989, too. The method of tissue culture for adult birch took place according to normal routine practice using vegetative buds as tissue material for the micropropagation of birch in the laboratory (Ryynänen and Ryynänen 1986). WPM (Lloyd and McCown 1980) with BAP 4,4 µM was used for cultivation. Before rooting the shoots were subcultured in MS medium (Murashige and Skoog 1962) made to half-strength with 2,2 µM BAP and 2,85 µM IAA. The rooting of shoots took place in the same MS medium without any growth regulators. The rooted shoots about 20 mm high were potted and the seed was sown in peat:perlite mixture (1:2) at the same time, in the middle of April. Grafting was done by using one-year-old and 30 cm high seedlings as rootstocks. The scions from each mother tree were grafted onto an open-pollinated progeny of the same tree. The material was grown in an unheated greenhouse under natural light during spring–summer 1989 and in an open nursery during winter 1989–90. In May 1990 the plants were transplanted into 8 liter pots with fertilized peat in the greenhouse.

The experiment was established in a 50 m long, 16 m wide and 6 m high, polythene arched hall in June 1990. The growing conditions in the experiment were maintained as similar as possible to the conditions in a polythene greenhouse seed orchard (Pirttilä and Saarela 1989). The soil at the growing site of the experiment was glacial till, covered with a 30 cm layer of peat. The peat was limed during the establishment of the trial and fertilized thereafter with NPK fertilizer (7-5-15) in August 1990 and 1991 as well as with NPK fertilizer (10-7-14) in spring 1991–93. Natural light was used and the temperature during the summer was maintained at +25 °C.

The experiment was carried out using 10 different genotypes (clones), each represented by

two micropropagated plants, two grafts and two seedlings originating from open-pollinated seeds of these clones. Of each plant material type typical representatives with good quality and equal size were selected for the experiment. The growing area was subdivided into two blocks according to the direction of sunlight. Groups of the different types of material were planted randomly in the blocks, so that each block included one individual/type of propagation/genotype. Thus, each material type was represented by 20 plants altogether.

The plants were topped to the height of 3.5 m in August 1991, after they had reached the ceiling of the polythene hall. Observations on seed production were made in 1990–1994, i.e., from the establishment of the experiment till the plants reached the age of five. Seeds were collected manually in the end of July. The seed production per plant was weighed. In 1993 a flock of siskins (*Carduelis spinus*) managed to get into the greenhouse and browsed a part of the seeds. The siskins came into the polyhouse from one end and started browsing from there on. Thus, the damage caused by the birds was distributed unevenly over the hall varying from 0–80 % per plant. The floral axes of the seed catkins remained on the trees after browsing of the seeds. The proportion of these empty axes of the total number of seed catkins was estimated. This was used as an estimate to the proportion of seeds lost, and finally the seed production was calculated based on the estimated percentage of the seeds lost.

An analysis of variance (general linear model) and Tukey's test was used to test the significance of the differences between the types of plant material, clones, blocks and plant material type × clone interaction. The SAS/STAT™ statistical package was used (SAS Institute Inc. 1989). A mixed model was applied using material type as fixed and clone and block as random effects and type III estimable functions. Before the analysis of variance, a SQRT -transformation was done to the seed production in 1993, whereas LOG-transformed values were used for the analysis of variance of seed production in 1992 and 1994.

3 Results

None of the plant material types flowered in spring 1990, when the experiment was established, but at the age of two years, in spring 1991, the grafts flowered (Table 1). Female catkins developed on some of the grafts of two of the clones (E5201 and E5396), which then went on to produce ripe seed. The total amount of seed produced by the graft material was 4.95 g.

In 1992, seed production occurred on all three plant material types (Table 1, Fig. 1).The seedlings produced the highest number of seeds per plant and the grafts the lowest. The differences between the material types were not, however, statistically significant, but the interaction between plant type × clone was statistically significant (Table 2). Seed production was observed on all plants except one graft of clone E5387 and one seedling of clone E5385.

In 1993, when the experiment was four years old, the material of all types produced a high amount of seeds per plant. Seed production of all three material types was more than ten times higher than that of the previous year (Table 1, Fig. 2). Production of seeds per plant was the

Table 1. Seed production (g/plant) of seedlings, micropropagated plants and grafts of silver birch in 1991–94. Different letters indicate significant differences between plant material types (Tukey's test, $p < 0.05$).

Year (Age)	Material type Seedling x̄	Seedling s.d.		Micropropagated x̄	Micropropagated s.d.		Graft x̄	Graft s.d.	
1991 (2)	0			0			0.2	0.9	
1992 (3)	72.4	97.2	a	66.7	74.8	a	26	41.9	b
1993 (4)	807.9	760.7	a	609.5	386.2	a	326	261.3	b
1994 (5)	14.1	36.3	a	13.6	32.1	a	16.4	23.5	a

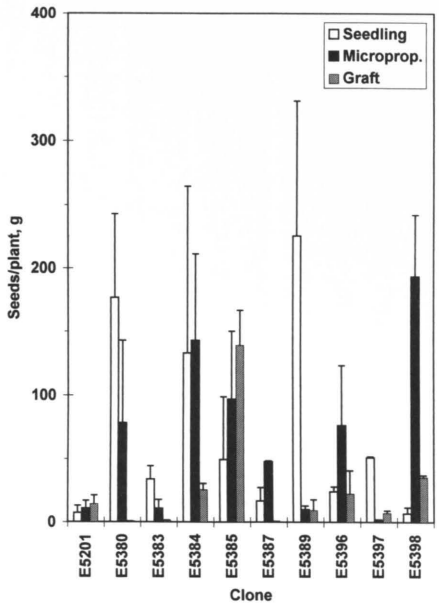

Fig. 1. Seed production of different plant material types and clones in 1992 at the age of three years. Each bar is the mean and standard error (s.e.) of two trees (n = 2).

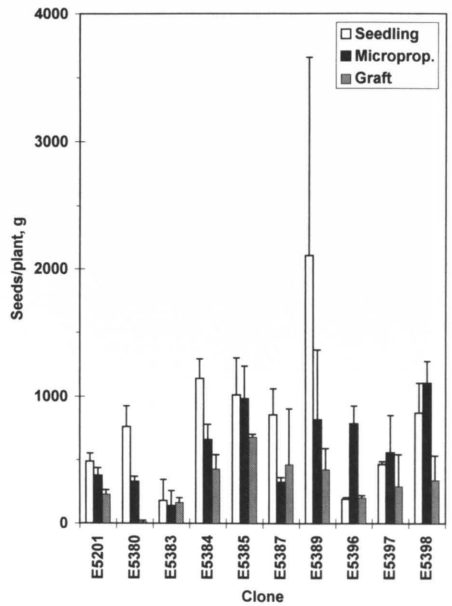

Fig. 2. Seed production of different plant material types and clones in 1993 at the age of four years. Each bar is the mean and standard error (s.e.) of two trees (n = 2).

Table 2. ANOVA table of seed production of different plant material types in 1992–1994.

Year (Age)	Plant material type	Clone	Block	Plant type × clone	Error
1992 (3)	F = 1.78 DF = 2 p < 0.1964	F = 1.91 DF = 9 p < 0.0898	F = 0.03 DF = 1 p < 0.8552	F = 3.07 DF = 18 p < 0.0035	DF = 29 MS = 1.42
1993 (4)	F = 8.65 DF=2 p < 0.0023	F = 3.38 DF = 9 p < 0.006	F = 0.49 DF = 1 p < 0.4877	F = 0.93 DF = 18 p < 0.5539	DF = 29 MS = 65.46
1994 (5)	F = 1.06 DF = 2 p < 0.3663	F = 1.63 DF=9 p < 0.1517	F = 0.7 DF = 1 p < 0.4112	F = 0.94 DF = 18 p < 0.5478	DF = 29 MS = 2.39

highest in seedlings and the lowest in grafts. The seedlings as well as the micropropagated plants differed significantly from the grafts. Variation among clones was also statistically significant. The interaction between plant material type and clone was, however, no more significant (Table 2). All plants, except one graft of clone E5380, produced seed.

In 1994, flowering and seed production of all three plant material types was very poor (Table 1, Fig. 3). No significant differences were observed (Table 2). No seed production was observed on 24 plants in the whole experiment.

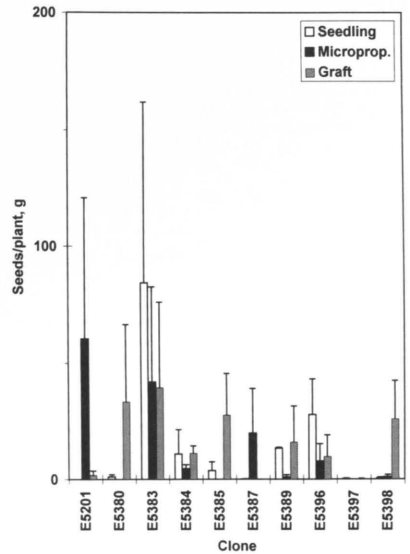

Fig. 3. Seed production of different plant material types and clones in 1994 at the age of five years. Each bar is the mean and standard error (s.e.) of two trees (n = 2).

4 Discussion

In our study the graft material started to flower at the age of two years, one year earlier than the micropropagated plants and seedlings. This might be explained by differences in maturity, i.e., grafts could be more mature than the micropropagated plants and seedlings and less rejuvenated than the micropropagated plants as discussed by Viherä-Aarnio and Ryynänen (1995). Rejuvenation of birch (*Betula* sp.) has been reported by Brand and Lineberger (1992). In their study, flowering ability was one of the first features to be lost during rejuvenation and one of the last to be regained with maturation. In the study of Brand and Lineberger (1992) micropropagated plants developed mature phenology more rapidly than seedlings, and they concluded that the level of juvenility regained by micropropagated plants may not be equivalent to that of the seedlings.

The delayed flowering of micropropagated birch compared to grafts we observed probably has no practical importance on total seed production since, in the first year, even the flowering of the grafts was low. In the following year, seed production of the micropropagated plants and seedlings was already more than two times that of the grafts. As reported by Viherä-Aarnio and Ryynänen (1995) the micropropagated plants and seedlings grew more vigorously and had bigger and more ramified branches than the grafts. Evidently the size and ramification of the branches has a considerable effect on seed production – the longer and the more ramified the branches are, the more there are potential sites for male and female catkins. According to results by Larsen and Higgins (1993), micropropagated apple trees can also overcome some effects of delayed early fruit bearing because of the generally larger tree size compared with grafts. Topping is used in birch seed orchards in order to improve the branching of the trees (Pirttilä and Saarela 1989). The plant material of this study was topped at the age of two. Topping may have affected the different material types in a different way. Before topping there were, for instance, significant differences between the material types in the number of branches, but after topping these differences no longer existed, since the grafts were able to increase their branch number more than the two other types (Viherä-Aarnio and Ryynänen 1995).

Birch seedlings may start flowering at the age of two or three in unheated greenhouses (Kärki 1976). Through increasing the photoperiod, regulating temperature, increasing CO_2 concentration, fertilization, irrigation and increasing air humidity, flowering can be induced even as early as at the age of eight months (Longman and Wareing 1959, Kärki 1976, Holopainen and Pirttilä 1978). Micropropagated plants can probably also be induced to flower earlier through such intensive treatments.

Interestingly, the plant material type × clone interaction was significant in the third year, i.e., different genotypes behaved differently when used as different material types (Table 2). This might indicate that the speed of maturation or the degree of rejuvenation varies with the genotype. At the age of four and five the interaction was no more significant.

On apple trees clonal rootstocks are often used and the rootstock × scion interaction has a very significant effect on the yield of apples (Webster et al. 1985, Zimmerman and Miller 1991, Larsen and Higgins 1993). The rootstock × scion interaction on birch is not known, but in order to minimize its possible effect on seed yield in this study, the open-pollinated progeny of the grafted trees were used as rootstocks.

In 1993, when the seed production of natural birch stands in Finland was extremely high (METLAn siemensatoennuste 1994), it was very good in our experiment as well. The micropropagated plants produced two times more seed than the grafts and 75 % of the seed crop of the seedlings. This suggests that micropropagated plants in seed orchards would be better seed producers compared to grafts when measured as the average seed production of the different plant types. The variation between different clones was, however, significant, a fact which affects the genetic constitution of the seed. It should also be kept in mind that the seedlings did not represent exactly the same genotype as the grafts and micropropagated plants, since they were open-pollinated progeny of the grafted and micropropagated trees.

Variation in seed production within different plant material types was the highest in seedlings (coefficent of variation 94 %) and the lowest in micropropagated plants (63 %), the grafts being intermediate (80 %). This suggests that the proportion of different genotypes in the total seed crop would be more even and the genetic quality of the seed would be more homogeneous when using micropropagated material instead of seedlings or grafts. This study was, however, made with limited material (ten clones), and it is quite obvious that more studies are needed concerning the clonal variation of seed production as well as male flowering. Further studies are also needed concerning the physiological quality (germination, seed weight etc.) of the seed produced by different plant material types.

The phase of rich flowering and seed production of seed orchards in polythene greenhouses has been shown to last for about six years, whereafter flowering decreases (Tyystjärvi and Pirttilä 1984). The very poor flowering and seed crop in 1994, at the age of five, was not, however related to age. Seed production in 1994 was also very poor in natural stands of birch (METLAn siemensatoennuste 1994). Therefore the low seed production we observed was rather the result of normal between year variation. Observations on male catkins at the end of summer 1994, suggest that flowering next year will again be very abundant. It will be important to follow our experiment a few more years in order to gain more information on the length of the production time and the total seed crop as the different plant material types age.

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