Performance of Micropropagated Plants of Silver Birch (*Betula pendula*) in a Field Trial

Anneli Viherä-Aarnio

Viherä-Aarnio, A. 1994. Performance of micropropagated plants of silver birch (*Betula pendula*) in a field trial. Silva Fennica 28(4): 283–288.

Micropropagated and seed-born plants of silver birch (*Betula pendula* Roth) were compared for survival and growth in a field trial at the age of six years. Three clones for micropropagation were selected from open-pollinated progenies of selected southern Finnish plus trees at the age of 17 and 20. The three seed-born lots were of southern Finnish stand origin. The best two lots of the experiment as regards the height and diameter growth at the age of six were the clones. The best of these differed significantly from the best growing seed-born lot. The weakest lot of the experiment was also a clone, which was clearly slow-growing with a dense and bushy-like crown. Survival of the material was high (mean = 94 %), and there was no damage caused by voles and elks for example. The results clearly show, that the selection of material for clonal propagation should be done carefully. The clones should also be tested for performance in the field before propagation on a large-scale.

Keywords *Betula pendula*, clones, forestry, tissue culture, seedlings.
Author's address The Finnish Forest Research Institute, P.O. Box 18, FIN-01301 Vantaa, Finland **Fax** +358 0 857 2575 **E-mail** anneli.vihera-aarnio@metla.fi
Received October 14, 1994

1 Introduction

Research on tissue culture of birches (*Betula* spp.) has been done with a number of species and varieties as reviewed by Meier-Dinkel (1992). Micropropagated birch plants which could be established in soil have been obtained with *B. pendula* (Huhtinen and Yahyaogly 1974, Chalupa 1981a, Särkilahti 1988), *B. pendula* f. *purpurea* (Simola 1985), *B. pendula* var. *carelica* (Ryynänen and Ryynänen 1986), *B. platyphylla* var. *szechuanica* (McCown and Amos 1979), *B. pubescens* (Chalupa 1981b), *B. papyrifera* (Minocha et al. 1986) and *B. maximowicziana* (McCown 1989). Vegetative propagation of mature trees is an important aim from the point of view of forest tree breeding. Micropropagation of compelete plants from mature trees has been reported on *B. pendula* (Cha-

lupa 1981a, Särkilahti 1988) and on *B. pendula* var. *carelica* (Ryynänen and Ryynänen 1986).

The first steps towards large-scale production of micropropagated material were the *in vitro* propagation of several hundred plants of juvenile *B. platyphylla* var. *szechuanica* reported by McCown and Amos (1979) and of mature *B. pendula* var. *carelica* reported by Ryynänen and Ryynänen (1986). In Finland the application of tissue culture in practical forestry started in 1987, when three companies established a joint project to micropropagate birches on a larger scale. In this project, 300 000 micropropagated plants of 30 different genotypes were produced in 1989 (Jokinen et al. 1989) and the first clonally propagated birch plants were used for practical forest cultivation in spring 1989.

There was and still is, however, very little experience on the performance of micropropagated birch plants in field conditions. McCown and Amos (1979) followed the height growth of micropropagated plants and seedlings of *B. platyphylla* var. *szechuanica* in the field for one season and showed that both had identical growth rates in the spring and summer, but the micropropagated plants stopped growth one month earlier than the average seedlings. In the study of Jokinen and Törmälä (1991) the performance of the micropropagated plants of *B. pendula* during the first season in the nursery was slightly better than that of conventional seedlings. Very few results on field testing of micropropagated material in the forest have been reported. Meier-Dinkel (1992) compared three *in vitro* propagated clones of *B. pubescens* in a field experiment in the lowlands of North Germany. The performance of the micropropagated material was good, since at the age of four, the height of the plants reached more than three meters. Two more field experiments, laid out in the mountain range area, containing two clones of *B. pendula* and three of *B. pubescens* as well as six clones of *B. platyphylla* var. *japonica* × *B. pendula* and one clone of resiprocal hybrid were reported by Meier-Dinkel (1992). The growth of *B. pubescens* and *B. pendula* clones was rather weak, whereas the hybrid clones showed superior growing capacity compared to the clones of the indigenous pure species.

The aim of this study was to compare the performance of micropropagated and seed-born plants of silver birch under field conditions similar to conditions in practical forest regeneration. Preliminary results are presented from one of the first field tests established for comparison of micropropagated plants and seedlings in Finland.

2 Material and Methods

The material of the experiment (No 1336/1) consists of three clones and tree seed-born lots (Table 1). All the clones were selected from open-pollinated progenies of southern Finnish plus trees. The clones were selected from 17 and 20-year-old plantations, which all grow near to the city of Imatra, south-eastern Finland (61°14'N, 28°50'E). Selection was done in summer 1988 by the personnel of Enso-Gutzeit Company, and these clones were among the very first ones selected for the micropropagation project of the company. The original plus trees, E 173 Imatra, E 2818 Valkeakoski (61°12'N, 24°00'E) and E 4052 Sysmä (61°21'N, 25°40'E) , i.e., the mother trees of the clones in the experiment, were selected by the Forest Research Institute in 1948, 1964 and 1971, respectively, according to the routine practice for plus tree selection using state of health, growth and stem quality as selection criteria.

Two of the seed-born lots in the experiment represented southern Finnish seed collection stands. The Taipalsaari stand (61°21'N, 28°15'E, 85 m asl) is situated in south-eastern Finland and the Lieksa stand (63°19'N, 30°01'E, 130 m asl) is some 200 km further north. The third seed-born lot was seedlings grown from open-pollinated seed collected from a full-sib family, E 1970 Kangasala × E 1980 Nummi-Pusula, which is growing in a field trial at Rautalahti experimental area (62°08'N, 25°43'E, 85 m asl) close to the city of Jyväskylä.

Micropropagation of the material was done by the Kemira and Hortus companies according to the axillary micropropagation method with no callus phase (Jokinen, pers. comm.). Vegetative buds were used as tissue material. WPM (Smith and McCown 1982) supplemented with 2 % sucrose, 2 mg l^{-1} BAP and 0.05 mg l^{-1} NAA was used for initiation of the culture. Explants with induced axillary buds were divided and transferred to WPM with 2 % sucrose and 0.5 mg l^{-1} BAP. Rooting of shoots took place in WPM with 2 % sucrose and IBA 0.05 mg l^{-1}. The rooted plantlets were transferred to pots with peat:perlite (1:1) mixture in the greenhouse and acclimated in 98 % humidity and +20 °C temperature for two weeks. The micropropagated plantlets were grown further at the Enso-Gutzeit Ukonniemi nursery near Imatra. In May 1988 the plantlets were transplanted to 3 dl pots with limed and fertlized peat and grown in a plastic greenhouse with mist irrigation for 6–7 weeks. After that they were transferred to an open nursery. The seedling material was sown to 3 dl pots with limed and fertilized peat in a plastic greenhouse in May 1988 and transferred in an open nursery in the middle of the summer. In May 1989 the one-year-old micropropageted plants and seedlings were transferred to the field trial. At that time their average height was about 70–80 cm.

Table 1. Description of the material in experiment 1336/1 at Vaajakoski.

Exp. lot	Material type	Clone or seedlot	Origin
1	clone	KL 2 M	Selected from open pollinated progeny of plus tree E 2818 Valkeakoski (61°12'N, 24°00'E, 90 m asl)
2	clone	KL 4 M	Selected from open pollinated progeny of plus tree E 4052 Sysmä (61°21'N, 25°40'E, 90 m asl)
3	clone	KL 1 M	Selected from open pollinated progeny of plus tree E 173 Imatra (61°15'N, 28°50'E, 100 m asl)
4	seedlings	P27-73-0992	Taipalsaari, plus stand 992 (61°21'N, 28°15'E, 85 m asl), stand seed
5	seedlings	P27-87-0001	Lieksa (63°19'N, 30°01'E, 130 m asl), stand seed
6	seedlings	P27-87-0005	(E 1970 × E 1980) open pollination in progeny trial 542/6 at Jyväskylä (62°08'N, 25°43'E, 85 m asl)

The experiment was established in the Jyväskylä municipality (62°15'N, 26°02'E, 140 m asl). The site was a moist upland forest site, *Myrtillus* site type according to the Finnish classification, and the soil was developed on fine sand till. The area was ploughed before planting. The experiment was established with one-year-old plants in spring 1989. A randomized block design with five blocks was used. Plot size was 14 × 14 m with 49 plants per plot at 2 × 2 m spacing. The total area of the experiment was 0.6 ha. The trial was surrounded by a shelterbelt of birch. The experiment was weeded and cleaned mechanically during the early development.

The trial was measured in September 1993. The height and breast height diameter (dbh) were measured and the number of branches were counted. The ratio between the shoot height and number of branches per tree (shoot height/branch number) was calculated in order to describe the branchiness. The statistical significance of the differences in survival was tested using non-parameter Friedman's two-way analysis of variance according to Kouki et al. (1990). A two-way analysis of variance and Tukey's test were used in order to determine the significance of the differences in height, diameter, branch number and shoot height/branch number ratio. The analysis of variance was performed on the plot means. The standard deviation (S.D.), which illustrates the within lot variation of each character, was calculated from all observations in the whole experiment within each experimental lot. The SAS/STAT™ statistical package was used (SAS Institute Inc. 1989).

3 Results

The average survival of the experiment was 94 %, and the average height of the plants was 333 cm (Table 2). There were statistically significant differences between the lots in all traits: surviv-

al, height, dbh, number of branches and shoot height/ branch number ratio (Table 2).

The best lot as regards the height growth was the clone KL 4 M, selected from the open-pollinated progeny of plus tree E 4052 from Sysmä (Table 2). It differed significantly from the best stand seed origin, Taipalsaari. Clone KL 2 M selected from the progeny of plus tree E 2818 Valkeakoski ranked the second as regards the growth. The weakest lot in the trial was also a clone, KL 1 M selected from the progeny of plus tree E 173 Imatra. The stand seed lot from Lieksa had the highest shoot height/branch number ratio, whereas the weakly growing clone KL 1 M had the smallest ratio. The performance of this clone in general was inferior, slow-growing and bushy-like.

4 Discussion

As the number of the lots in the experiment was limited, broad generalizations about the success of micropropagated material of birch cannot, of course, be made. It is, however, one of the first field trials established with micropropagated birch material in Finland. In general, the trial has succeeded well. This is mainly because it has escaped the most significant damage agents in young birch plantations – elks and voles. This is evidently due to its location in a populated area, close to roads and houses. It is, however, noteworthy that the survival of both the micropropagated and the seed-born material was high. The only lot with clearly lower survival compared to the others was the seedlings from open-pollination of the full-sib family E 1970 × E 1980.

Table 2. Survival, growth and branchiness of clones and seed-born lots in experiment 1336/1 Vaajakoski. Different letters indicate significant differences between the lots (Tukey's test, $p < 0.05$).

Exp. lot	Plant type	Clone number, origin	Survival, %	Height, cm (S.D.)	D.b.h., mm (S.D.)	Number of branches (S.D.)	Shoot height/ branch number, cm (S.D.)
1	clone	KL 2M E2818 Valkeakoski open poll.	94	367 (73) ab	21 (6.7) ab	43 (8.5) ab	8.7 (1.3) bc
2	clone	KL 4M E4052 Sysmä open poll.	98	383 (67) a	22 (6.7) a	49 (9.9) a	7.9 (1.0) c
3	clone	KL 1M E173 Imatra open poll.	93	271 (75) e	16 (6.5) c	41 (11.7) b	6.7 (1.5) d
4	seedlings	Taipalsaari plus stand seed	95	341 (63) bc	20 (6.8) ab	40 (8.1) b	8.5 (1.1) bc
5	seedlings	Lieksa stand seed	98	301 (67) de	17 (6.9) bc	32 (8.6) c	9.7 (1.7) a
6	seedlings	(E1970 × E1980) open poll. in Jyväskylä	84	330 (73) cd	19 (7.0) abc	36 (9.1) bc	9.3 (1.3) ab
Experimental mean			94	333 (79)	19 (7.1)	40 (10.9)	8.5 (1.6)
Analysis of variance							
Lot			F=3.95 DF=5 p<0.0093	F=24.32 DF=5 p<0.0001	F=6.38 DF=5 p<0.0011	F=14.47 DF=5 p<0.0001	F=23.38 DF=5 p<0.0001
Block				F=10.17 DF=4 p<0.0001	F=6.58 DF=4 p<0.0015	F=4.85 DF=4 p<0.0067	F=1.11 DF=4 p<0.3802
Error			MS=1.77 DF=24	MS=3.57 DF=20	MS=4.38 DF=20	MS=12.09 DF=20	MS=0.00 DF=20

Two of the clones had grown better than the seed-born lots from stands of local origin. Thus, in these cases the micropropagated material was able to grow normally and at least as well as the seed-born one. In the study of Meier-Dinkel (1992) the growth of micropropagated plants of *B. pubescens* in the lowlands of North Germany was good, since at the age of four the plants were more than three meters high. In another experiment in the mountain range area the growth of micropropagated plants of *B. pendula* and *B. pubescens* was much slower, which was explained by poorer growth conditions and sensitive response to transplanting of the 2-year-old plants (Meier-Dinkel 1992). Comparison to seedlings could not be made in that study, because the trials did not include seed-born lots (Meier-Dinkel 1992). In the study of McCown and Amos (1979) micropropagated plants and seedlings had identical growth rates in the spring and summer in the first year, but micropropagated plants stopped growth one month earlier than the seedlings. This resulted in the micropropagated plants having a smaller size than the seedlings. An explanation to this could be either physiological difference between micropropagated plants and seedlings, or clonal propagation by chance of a genotype, which goes dormant early in fall as discussed by McCown and Amos (1979).

The differences in growth among the lots are possibly caused by the average genetic differences between the origins, since the clones were not selected from same stands as where the seed-born lots come from. Thus, the results presented here do not give any indication of the genetic gain obtained by clone selection. The Taipalsaari seed stand is in the same geographical area as the clones. The slower growth of the Lieksa stand lot may be partly due to its more northern origin, and indeed it may be considered "too northern" to be used as a standard for southern Finnish clones. The open pollination of E 1970 × E 1980 from Jyväskylä, appears not to have much value as a seed-born standard lot. However, the full-sib family E 1970 × E 1980 itself (also known by the commercial name "JR-1") may have given better results than its progeny.

Jokinen and Törmälä (1991) followed the growth of four micropropagated silver birch clones and two seed-born lots during the first growing season in the nursery. The two seed-born comparison lots ("Lieksa" and "JR1") used by Jokinen and Törmälä (1991) were the same as used in this study (Lieksa stand and open-pollination of E 1970 × E 1980). Their slower growth compared to the clones could also be caused by the average genetic differences between the geographical origins as mentioned above. On the other hand, the best performing clone ("Micro 39") in the study of Jokinen and Törmälä (1991), which is the same clone as KL 2M in this study, was somewhat more southern than the other origins.

McCown and Amos (1979) reported that the micropropagated plants were highly uniform in growth and grade as compared to the seedlings. In this study the variation in height and diameter within the lots was about the same in both the micropropagated and seed-born material. In the number of branches the within lot variation was even somewhat higher in the clone KL 1M than the other lots. This clone expressed a variable growth habit including both normal plants and bushy-like plants with a dense crown. Its poor and variable performance could be due to somaclonal variation in the original selected individual of the clone or due to mutations or metabolic disorders occurred during the propagation process. According to Jokinen (pers. comm.) BAP used as a cytokinine may increase the formation of axillary buds, which could have caused the bushy-like performance. On the other hand, Jokinen and Törmälä (1991) reported earlier that there was no indication of variants among the about 400 000 plants of 100 genotypes produced through the axillary system. McCown and Amos (1979) observed only one visually abnormal shoot after propagation of hundreds of thousands of plants, indicating that the genetic stability of the culture remains high when using shoot tip culture and preformed meristems of the original explant.

The selection of the clones for micropropagation on a large-scale should be done very carefully and with tight selection criteria. It must be kept in mind that the clones in this trial represent the very first clones selected for micropropagation, and the selection was made in progeny plantations with quite subjective criteria. Later selections for clonal propagation of birch have

been made in progeny trials as a combined family-individual selection. Despite careful selection there is still a possibility of occurrence of variants and clones with unexpectedly bad performance, like KL 1M in this study. Due to this, it is important to test the clones in field trials before wide scale propagation and marketing.

Acknowledgements

I wish to thank Enso-Gutzeit Company for cooperation in initiating the experiment, i.e., for providing the experimental material, site and planting personnel and for early management of the experiment, as well as Tehdaspuu Company for later treatment of the experiment. I also wish to thank Kari Jokinen for valuable information concerning the micropropagation method, Marja-Leena Annala, Lea Siilin and Maria Boije af Gennäs for measuring the experiment and Sisko Salminen for typing the tables.

References

Chalupa, V. 1981a. In vitro propagation of birch (Betula verrucosa Ehrh.). Biologia Plantarum 23(6): 472–474.

— 1981b. Clonal propagation of broad-leaved forest trees in vitro. Communicationes Instituti Forestalis Cechosloveniae 12: 255–271.

Huhtinen, O. & Yahyaogly, Z. 1974. Das frühe Blühen von aus Kalluskulturen herangezogenen Pflänzchen bei der Birke (Betula pendula Roth). Silvae Genetica 23(1–3): 32–34.

Jokinen, K. & Törmälä, T. 1991. Micropropagation of silver birch (Betula pendula Roth) and clonal fidelity of mass propagated birch plants. In: Ahuja, M.R. (ed.). Woody plant biotechnology. Plenum Press, New York. p. 31–36.

— , Törmälä, T. & Virta, U. 1989. Clonal fidelity of mass propagated silver birch (Betula pendula Roth). In: Abstr, IUFRO Somatic cell genetics Working Party S2-04-07 and NATO Advanced Research Workshop, Woody plant biotechnology, Placerville, CA, Oct 15–19, 1989.

Kouki, J., Ranta, E. & Rita, H. 1990. Biometristen aineistojen analyysi SAS-ohjelmistolla. University of Helsinki, Department of Silviculture. Research Notes 66. 90 p.

McCown, B. 1989. Birch (Betula spp.). In: Bajaj, Y.P.S. (ed.). Biotechnology in agriculture and forestry. Vol 5. Trees II. Springer-Verlag, Berlin, Heidelberg, New York. p. 324–341.

— & Amos, R. 1979. Initial trials with commercial micropropagation of birch selections. International Plant Propagators Society Combined Proceedings. 29: 387–393.

Meier-Dinkel, A. 1992. Micropropagation of birches (Betula spp.). In: Bajaj, Y.P.S. (ed.). Biotechnology in agriculture and forestry. Vol. 18. High-tech and micropropagation II. Springer-Verlag, Berlin, Heidelberg. p. 40–81.

Minocha, S.C., Noh, E.W. & Kausch A.P. 1986. Tissue culture and and genetic transformation in Betula papyrifera and Populus tremuloides. Research and development Conf, Sept 1986, TAPPI, Technology Park/Atlanta, p. 89–92.

Ryynänen, L. & Ryynänen, M. 1986. Propagation of adult curly-birch succeeds with tissue culture. Silva Fennica 20(2): 139–147.

Särkilahti, E. 1988. Micropropagation of a mature colchicine-polyploid and irradiation-mutant of Betula pendula Roth. Tree Physiology 4: 173–179.

SAS Institute Inc. 1989. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 2, Cary, NC, USA. 846 p.

Simola, L. K. 1985. Propagation of plantlets from leaf callus of Betula pendula f. purpurea. Scientia Horticulture 26(1): 77–86.

Smith, M.A.L. & McCown, B.H. 1982/83. A comparison of source tissue for protoplast isolation from three woody plant species. Plant Science Letters.28: 149–156.

Total of 15 references

Reviews of Manuscripts 1994

The editors of Silva Fennica and Acta Forestalia Fennica have received invaluable assistance from experts who have provided reviews of manuscripts submitted to the journals. For reviews completed in 1994, the editors would like to extend their sincere thanks to the following scientists.

Eric Agestam
Arne Albrektson
Stig Andersson
Hartmut Bossel
Finn H. Braekke
J. Douglas Brodie
Lars Christersson
Phil Comeau
Joan E. Cottrell
Raymond L. Czaplewski
Harry Eriksson
Sten Gellerstedt
Michel Goulard
David Grigal
Matti Haapanen
Hely Häggman
Risto Häkkinen
Jan-Erik Hällgren
Björn Hånell
Heikki Hänninen
Håkan Hytteborn
Hannu Ilvesniemi
Markku Kanninen
Jochen Kleinschmit
Michael Köhl
Aune Koponen
Veikko Koski
Koen Kramer
Jari Kuuluvainen
Timo Kuuluvainen
Juha Lappi
Martin J. Lechowicz
Dag Lindgren
Kåre Lund-Höie
Alpo Luomajoki
Noel Lust
Annikki Mäkelä
Euan G. Mason
William J. Mattson
Jouni Mikola
Hugh G. Miller
Maureen B. Murray
Eero Nikinmaa
Jens Viktor Nørgaard
Markku Nygren
Risto Ojansuu
Göran Örlander
Juni Palmgren
John Pastor
Bengt Persson
Kurth Perttu
Bengt Pontén
Peter Poschen
Pertti Pulkkinen
D.G. Pyatt
Hannu Raitio
Juhani Rinne
Hannu Rita
Matti Rousi
Lars Rytter
Ola Sallnäs
Aila Särkkä
Outi Savolainen
John Sessions
Oddvar Skre
Erik G. Ståhl
Frits Staudt
Richard Stephan
Antti Uotila
Erik Valinger
Lauri Valsta
Elina Vapaavuori
Seppo Vehkamäki
Pirkko Velling
Martin Werner
Carl Johan Westman
A.G.D. Whyte
Claire G. Williams
Anneli Ylimartimo
Atsushi Yoshimoto

Submission of Manuscripts Manuscripts should be submitted in triplicate to Silva Fennica, Unioninkatu 40 A, FIN-00170 Helsinki, Finland. Detailed instructions to authors are printed in the first issue each year, and can be found on WorldWideWeb at URL http://www.metla.fi/publish/silva/SF-Instructions.html/. Offprints of Instructions are available on request.

Publication Schedule Silva Fennica is issued in four numbers per volume.

Subscriptions and Exchange Subscriptions and orders for back issues should be addressed to Academic Bookstore, Subscription Services, P.O. Box 23, FIN-00371 Helsinki, Finland, Phone +358 0 121 4430, Fax +358 0 121 4450. Subscription price for 1995 is 300 FIM (for subscribers in Finland 200 FIM). Exchange inquiries should be addressed to The Finnish Society of Forest Science, Unioninkatu 40 B, FIN-00170 Helsinki, Finland, Phone +358 0 658 707, Fax +358 0 191 7619.

Statement of Publishers Silva Fennica has been published since 1926 by The Finnish Society of Forest Science. From 1994, the journal is published by the Finnish Society of Forest Science and the Finnish Forest Research Institute. The Finnish Society of Forest Science is a nonprofit organization founded in 1909 to promote forest research. The Finnish Forest Research Institute, founded in 1917, is a research organization financed by the Ministry of Agriculture and Forestry.

Abstracting Articles in Silva Fennica are abstracted and indexed in Agrindex, Biological Abstracts, Current Advances in Ecological Sciences, Current Advances in Plant Sciences, Ecological Abstracts, Forest Products Journal, Forestry Abstracts, International Bibliography of Periodical Literature, Life Sciences Collection.

SILVA FENNICA Vol. 28(4), 1994

The Finnish Society of Forest Science
The Finnish Forest Research Institute

Special issue on Adaptation of Tree Breeding to Changing Circumstances and Demands

ISSN 0037-5330

Cover: Jarmo Koivunen