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Vegetative propagation of conifers for enhancing landscaping and tree breeding

Proceedings of the Nordic meeting held in September 10th-11th 2008 at Punkaharju, Finland

Tuija Aronen, Teijo Nikkanen and Tiina Tynkkynen (eds.)

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Abstract
The Nordic meeting "Vegetative propagation of conifers for enhancing landscaping and tree breeding" was held at Punkaharju, Finland, in September 10-11th , 2008. The aim of the meeting was to share ideas and experiences among researchers, tree breeders, and commercial plant producers /nurseries, together with customers acting in landscaping or in forestry on the potentials and challenges in vegetative propagation of the Nordic conifers. There were altogether 54 registrated participants representing different stakeholders from six European countries.The meeting was hosted by Metla - Finnish Forest Research Institute, Punkaharju Research Unit, and kindly supported by GENECAR, Nordic Centre of Advanced Research in Forest Genetics and Tree Breeding, and The Foundation for Forest Tree Breeding in Finland. The information presented by the speakers during the meeting sessions, as well as by some of the posters is collected into this proceedings publication in the form of extended abstracts. The topics discussed are the following: I) Tree breeding framework, II) Landscaping framework, III) Technical issues – grafting and rooting of cuttings, and IV) Technical issues – potentials of tissue culture, and V) Collaboration and future sights containing also information on funding possibilities. In addition, the outcome of the group discussions focusing on the topics "How to facilitate the use of conifers as ornamentals?" and "Development of vegetative propagation techniques" is shortly reviewed.

Keywords
conifer, clonal testing, grafting, ornamental, rooted cuttings, tissue culture, tree breeding, vegetative propagation

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Contents

Preface

It was a great pleasure to host the meeting “Vegetative propagation of conifers for enhancing landscaping and tree breeding” at Punkaharju, on 10th –11th September, 2008. Organisation of the meeting was a continuation of both national and international discussions on the significance and great potentials of vegetative propagation in tree breeding and production.

The meeting was opened by Research Director, Prof. Pasi Puttonen from Finnish Forest Research Institute, and there were altogether 54 registrated participants representing different stakeholders from six European countries. Although the topic of the meeting, being focused on conifers, is challenging, we think we achieved the goal: During the meeting we were able to share ideas and experiences among researchers, tree breeders, and commercial plant producers /nurseries, together with customers acting in landscaping or in forestry. Also new initiatives for further collaboration and networking were presented and discussed.

The information presented by the speakers during the meeting sessions is collected into this proceedings publication in the form of extended abstracts of their talks. In addition to the talks given by the participants, some of the posters presented are included. The presentations were divided into the sessions on I) Tree breeding framework, II) Landscaping framework, III) Technical issues – grafting and rooting of cuttings, and IV) Technical issues – potentials of tissue culture. The last session in the meeting was V) Collaboration and future sights, that included information on funding possibilities together with group discussions. There were two discussion groups: one focusing on the topic "How to facilitate the use of conifers as ornamentals?" and the other one on the topic "Development of vegetative propagation techniques". The outcome of the discussion session can be summarised as the following:

The group discussing about how to facilitate the use of conifers as ornamentals had a clear outlook that there is a strong need for exotic looking and hardy new material for landscaping in Nordic Countries and Baltic Area. At the moment the conifer material for ornamental purposes is rather limited, there is not very much possibilities you can choose from, and even more serious problem is that most part of the material is produced in Central Europe and is therefore often not hardy enough to use in harsh Nordic conditions. The group, consisting of participants from Metla, Taimityllilä Nursery and City of Helsinki, and Kalsnava Arboretum, Latvia, agreed that we have to increase our own production of ornamental conifers. But there are many problems to be solved before large-scale production of new, hardy ornamental conifers can start. We have to find the hardy and desired varieties to be produced, market them, and to solve technical problems concerning vegetative propagation of these new ornamental options for landscaping. For all this the co-operation is needed, and the group decided to find out possibilities for launching a Nordic-Baltic collaborative project on the topic.

The group focusing on the development of propagation techniques was unanimous on the need of good biological understanding of the key processes of plant development in order to be able to manipulate it by vegetative propagation methods. Especially ageing as a phenomenon affecting propagation ability would need more basic research e.g. through gene expression and hormone studies, but also the influence of the environmental factors was recognised. It was stated that the common problems with rooting and plagiotrophisim are a consequence of predetermined development earlier in explants (cutting or seed embryo) life, and the key question is how to change predetermined fate of the plant cells or tissues? The group concluded that the development of propagation techniques should be combined with the research efforts addressing the basic development of plants both on genetic and physiological level. Continuation of this discussion and building of networks was expected to proceed e.g. through becoming European TREEBREEDEX activities. In addition, a new IUFRO unit "2.09.02 – Somatic embryogenesis of forest trees" coordinated by Yill-Sung Park (Canada) has recently been launched, and will surely facilitate networking in this research field. Participation of the Nordic researchers in these activities is highly encouraged.

Finally: Vegetative propagation is a way to utilise both natural and man-made innovations, no matter if these are mutations found in forest, or born as an outcome of a long-term breeding programme. We believe that there is a lot we can do together to get all the potentials of vegetative propagation fully exploited to benefit both landscaping and tree breeding. We hope the meeting "Vegetative propagation of conifers for enhancing landscaping and tree breeding" together with this Proceedings publication to be useful for everybody interested in vegetative propagation of conifers, and also act as a step towards building active collaborative networks among professionals and friends.

Participants on the meeting venue Lusto's inner yard, 11th September, 2008 – except some early birds who escaped before the group photo. (Photo: Timo Kilpeläinen)

Acknowledgements

The meeting was hosted by Metla - Finnish Forest Research Institute, Punkaharju Research Unit, and kindly supported by GENECAR, Nordic Centre of Advanced Research in Forest Genetics and Tree Breeding, and The Foundation for Forest Tree Breeding in Finland. We sincerely thank our sponsors for their valuable support, as well as the meeting venue Lusto, The Finnish Forest Museum, for good collaboration. We also would like to acknowledge all the people involved in organising the meeting and in preparation of these proceedings. Most important, however, are the contributions of all the participants. Thank you all for your personal input for making the meeting interactive and inspiring, and for sharing your knowledge and experiences by these proceedings.

February 16th, 2009

Tuija Aronen Teijo Nikkanen

Working Papers of the Finnish Forest Research Institute 114: 08–15

A way to utilise the advantages of clonal forestry for Norway spruce?

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It is suggested that vegetative propagation of offspring from the genotypes with the highest breeding values is an efficient way of harvesting genetic gain. This may be at least 20% superior to stand seeds (only half of Swedish spruce plantings are with seed orchard seeds). Controlled crosses may have a similar superiority even to a recently established elite seed orchard over the life time of the seed orchard, as the latest breeding material can be used for controlled crosses. The additional gain for forestry with tested clones seems to be less than 10% compared to controlled crosses, but this option is not technical available today, as rooted cuttings require juvenile stock plants and the SE technique is not yet mature.

Keywords: rooted cuttings, somatic embryogenesis, full sib crosses, controlled crosses, family forestry

Repeated efforts in Sweden and elsewhere to use tested clones of Norway spruce in commercial clonal forestry have been going on for forty years, but have failed, and new efforts are commercially unsafe. Mono-clonal forestry of e.g. Norway spruce is not well-tested enough to head for a major fraction of the plant market in the near future; it seems expensive; it has been frustratingly difficult to maintain clones juvenile during tests and propagation, much technical and biological development seem needed and there are legal; administrative and social problems to get monoclonal forestry accepted at a major scale, thus commercialisation should head only for a minor segment of the market. It is a much more cost-efficient and safer way to get a better forests over the whole cultivated area to see to that the seed orchard program is optimised and seed orchards are efficient than to head for vegetative propagation. But once this is done or on the way, some foresters want still more. The seed orchard situation in Sweden is rather good, it could be better, but it is developing well enough to consider methods to get still higher gain a limited part of the attention.

Vegetative propagation of seeds from controlled crosses with selected tested parents is a way to get most of the potential advantages of clonal forestry which has been advocated for long (e.g. Lindgren 1977). It is possible to get it implemented with fine-tuning of available techniques, without discounting major new break-throws. Implementation may be justified for a limited fraction of the forest if there is a will to make a large but justified investment and take some extra trouble and risk for a substantial gain. By choosing the very best parents with the top breeding-values for the desirable characters, a considerable higher gain than with seed orchards is possible, and by using recent measurement results for the parent selection a long delay in a seed orchard to utilize the gain is avoided. By controlled crosses pollen contamination and inbreeding, which reduces the seed orchard gain, can be eliminated. Controlled crosses can be custom tailored and head for more specific targets nearer in time than seed orchards. Most of the gain is in the controlled cross. Clones originating from controlled crosses are tested and a gain can be done above that of the original cross, but that is reflected as better parents to cross and not a real advantage with tested clones. The use of rooted cuttings is facilitated if the first cycle of stock plants can be done by Somatic Embryogenesis. Cryo-storage of the seed genotypes as cell-cultures will assure safer access of material to propagate. If those techniques are applied for those purposes, it means a considerable step and a need experience for the use SE plants of tested clones directly in the forest. The controlled cross can be seen as the first step towards forestry with tested clones for those who believe in that. “True” commercial clonal forestry using copies of field tested clones at an acceptable price is still not an option without discounting considerable future progress in technology.

Fig. 1.: The gain is in the seeds! The major gain of vegetative propagation seems to be to multiply seeds with superior parents, not tested clones. (Photo Anders Fries).

Gain estimates

Detailed gain calculations are long, tedious and based on many specific assumptions and therefore very specific and difficult to translate to general cases. Here rough estimates are made instead, more details about factors considered and how they are considered and methods and values used is mainly in Rosvall et al. (2002) and something in Weng et al. (2008), and Danusevicius & Lindgren (2002).

Plus tree seed orchards and amplified crosses of the best tested plustrees

The gain comparison is summarized in Table 1. Possible provenance effects are not considered or rather, the comparison is with stand seeds of comparable provenance. The Swedish seed orchards composed or untested plus trees give a gain of approximately 10% composed of selection gain at plus tree selection 8 percent, which is higher than Swedish estimates but epigenic effects are neglected. A gain by heterosis or release of inbreeding as plus trees are not related by 2%. The realised gain is reduced as a share of forest production in cultures originates from naturals, this is not considered. The plus trees have been progeny tested and the new seed orchards are established with selecting progeny tested clones with high breeding values. For the seed orchards it is assumed they are constructed by selecting the best 20 clones out of a number of tested trees and the corresponding crosses are made among the best 6 clones of the same number of tested trees. It is somewhat debatable how many trees where are (actually it is usually clone-testing nowadays for Norway spruce) and how well they are tested but the estimated gain is rather well documented (Rosvall et al. 2002). The selection intensity will be higher when selecting families, it the staring number was 200 it would be 30% higher which is assumed. The larger flexibility of controlled crosses is not given a value. There is a considerable time advantage of the controlled cross. A Norway spruce seed orchard has an economical life time of 40 years and the average plant appear on then market more than 30 years after the selection of the clones for seed orchard (Moriguchi et al. 2007). The average time from initiation of a crossing program till the cuttings are sold may be 6 years. That means that crosses are much closer to the breeding population. The figures here would indicate 24 years difference. It takes time to reach sexual maturity but that time can be used for longer genetic testing and is not lost. The gain per year in Norway spruce breeding is estimated to 0.5 percent per year of improvement for 20 years cycles, but here a more moderate estimate of 0.4% per year (somewhat higher than the figures by Danusevicius & Lindgren (2002) suggest, but lower than Skogforsk estimates) is used meaning about 24 years of breeding which is expected to give an extra about 10% gain.

Table 1. Gain comparison between seed orchards with selected clones and crosses among selected clones. The reference level is unselected clones from a stand.

Factor	Elite plus tree seed orchards	Crosses among elite plus tree clones
Selfing	0	+1
No mating among relatives, Gain by selecting plus trees.	+10	+10
Gain by selecting parents or clones among tested plus trees	+15	+19
Pollen contamination	-5	0
Crosses are closer the breeding population that seed orchards	0	+10
Gain compared to stand seeds considering selfing, initial plus tree selection, no relatives, gain by selection among tested clones and pollen contamination	20	30
Gain compared to stand seeds over the expected life time of the seed orchard	20	40

The values from Table 1 indicates that the gain in using crosses is 30% compared to stand seeds, and 20 percent compared to elite seed orchards. However, estimates are usually unsafe and have uncertainties and differ among situations, so I reduce them a little in the summary.

Added gain from using selecting tested clones instead multiplying their offspring

Once plants from somatic embryogenesis become cheaper than plants from rooted cuttings, it is little doubt SE plants will gradually take over the market from rooted cuttings, and the share on the market of tested clones will increase, as tested clones will be superior to mass-multiplicated families. But the additional gain maybe lower than many believe or trust decisions on. If clones are tested and preserved in juvenile form by somatic embryogenesis, they can be multiplied, but this is not done today. Tested clones of Norway spruce are kept in a form so they can be crossed at least during some time window as an integrated part of long term breeding in Sweden.

- Dominance variation and epistasis variation adds to gain if selected clones are planted instead of used as parents. The dominance variation in reasonable old experiments seems often to be around a quarter of the additive, implying that the dominance gives a contribution to selection gain, maybe 2% additional genetic gain to clonal forestry over CP forestry (gain is proportional to root of genetic variance, if variance increases by a factor 0.2 by non-genetic variation, gain increases by a factor 0.1). As dominance and epistasis variation are low, clone testing is sufficient to estimate breeding values and the gain in the additive variation, it is probably no good idea to reproduce tested families (Weng et al 2008) compared to crossing the best recently tested clones, because of the time and effort it takes to test a family compared and make use of the rather small extra gain added above the breeding value of its parents which is available without testing the family.
- There is a considerable genetic variation within in a full sib family, if the best tested clone is selected a considerable extra gain can be made compared by the corresponding CP (controlled pollination) cross. That variation is used in Swedish long-term breeding and creates parents which can be used for crosses to maintain the superiority of family forestry. If SE becomes operational, this within family clones can be multiplied as tested clones, but it is a time delay compared to family forestry crossing parents of the previous generation.
- If only the best clones were planted, the selection intensity would have been slightly higher for clonal forestry than for parents, but for Norway spruce in the foreseeable future mixtures of several clones and the similar requirements of non-relatedness as for family forestry are envisaged. Further on, it seems that many tested clones will not be found suitable for vegetative propagation. Thus the selection intensity for use as tested clones will not be higher than for use as parents.
- For long-term breeding of Norway spruce in south Sweden, the progress (made by within family selection) seems to be 8 percent production in a breeding cycle which takes 20 years, of which field testing is 15.
- It takes some years to get seeds and carry out the vegetative propagation with CP-seeds, for clonal forestry this might be started faster after testing, Thus clonal forestry is closer to the breeding population than the same clones used as parents, and that may give an advantage of say 3% (=7-8 years genetic progress in the breeding population).
- Thus clonal forestry may raise forest production about 5 percent above CP-forestry.
- CP-forestry requires sexual maturity; above I assumed 15 year old tests. If younger tests are used the time advantage is larger, but on the other hand the gain is lower and more doubtful. But considering this and that clonal forestry can be a bit opportunistic in entering material where prospects are best, it may be more fair to estimate the possible superiority to 7%.

More arguments against deploying single well-tested clones ("varietal forestry"):

- Superior clones may get trade identification and brands and owner rights following legal procedures. They are then likely to stay in the market even after they have been genetically outdated and passed their "best before" date. The market forces may encourage dominance of a few clones used over long time, even if it is not the best option for forestry.
- Testing and maintaining clones cost. If that is charged on a small market, it may mean high additional costs per plant sold.
- It is argued that clones are able to combine different characters; I suggest different factors can usually be combined into a single index and mainly dealt with as a single character. Thus this does not look as a major advantage for well-tested clones.
- Different clones are differently difficult to multiply vegetatively, thus the best tested clone may not be worth the added plant production cost.
- If SE-clones are tested, the cost of starting up a clone is considerable and thus the selection intensity may be considerable lower than with rooted cuttings. Maybe only 5 clones among 100 cell-lines started develop into clones easy to multiply.
- Somaclonal variation is likely to exist and increase during the life time of clones. Clones are likely to change performance during testing and use. That includes mutations. The risk of such changes seems smaller if clones live for as short time as possible.
- Clones quite likely accumulate diseases and genetic scrap. The argument why cloning is less common in animals is health considerations rather than diversity.
- The life time - and thus life-time associated problems - of clones become longer the better tested they are.
- A monoclonal stand is unsuitable for seed collection and natural regeneration (also in the neighbourhood)
- Worries about monoclonal forestry will limit the legally permitted and publically acceptable areas of vegetative propagation in forestry with Norway spruce and easily cause a legal limbo. Family forestry sounds more attractive to public and authorities.
- In terms of production value of long rotation major species I would recommend withdrawing around 2% of production for monoclonal culture for lack of diversity when making comparisons.
- Conclusion: Family forestry mixing two families seem unproblematic compared to a single tested clone from the forestry point of view.

SE for the first cycles?

- The logistics of artificial crosses become a major problem to get beyond some million cuttings annually.
- If SE makes the first cycle(s) in multiplication and cuttings just a last amplification by a factor 40-100, a high cost of each SE-plant is acceptable, the quantitative seed need would not be a problem, seeds can be blown up to any quantity and stock plants need not be shared many seasons. This is used for Sitka spruce on Ireland, where SE plants are multiplied by a factor 150.
- SE does not succeed in always and some are difficult to propagate. This matters less, when mass propagation of seeds rather than clones is important. Different number and success of different lines does not cause a major problem.

- SE can be stored, that means that suitable material is always available for mass multiplication and the dependence on that crosses can be made regularly is much reduced.

Genetic diversity

Absence of genetic variation may offer advantages:

- Homogeneity is an advantage for the market, for the forest manager and for the plant producer;
- If the rotation time is short, and the land controlled by the company owning one large mill, the characteristics can be fine-tuned to the end use;
- If rotation time is short clones can be changed when found sensitive or susceptible.

The most pessimistic risk scenarios expressed some decades ago seem not supported by the accumulated experience today.

Genetic variation got advantages:

- Different genotypes will utilize the different micro-niches on a site better together
- Diseases and pests are likely to make less harm in a diverse stand
- Diversity makes it possible to expand on what becomes the best share of the trees when they become older.
- The environments are variable and unpredictable, genetic variation contributes to that at least a share of the trees will be adapted to future conditions and thus give a higher stability.
- A genetically uniform stand may offer fewer niches for other living beings.
- Plants which propagate by cloning in nature typically have 3-15 varieties, indicating that there often is an advantage with clone mixtures.
- A mix of two full sib families is likely to be as variable as a natural stand from most relevant aspects.
- A genetically variable stand is a better seed source.
- More likely to get public acceptance and green certification;

The advantages of absence of genetic variation are probably small for a species like Norway spruce;

- The rotation time is long.
- The end use of the harvest is unpredictable.
- A felled stand is currently a small unit for the end user, thus homogeneity of individual stands is unimportant.
- Much of the harvest comes at thinning.
- A clone forest in boreal conditions may not become very uniform even when its genetics is uniform.
- The environments of plantations are variable and unpredictable;
- The sites are not much homogenized by management.

- The crop stays for a century, a mistake remains almost forever.

For Norway spruce in Sweden, the main line in the foreseeable future should be diversity, even when vegetative propagation is utilized. This may change but not until vegetative propagation is actually used for planting in some million copies over some years.

Concluding suggestion about clone number and relatedness

I suggest heading for mixtures of clones (mass-multiplied crosses) from full-sibs, which have near as much gene diversity as two unrelated full sibs. The gene diversity could more pedagogically be expressed as status number, which is half the inverted value of gene diversity. A large full sib family has status number 2 (all genes from both parents are where). But 6 clones from a full sib family catches most genes from the parents and has status number 2*6/(6+1)=1.7. Two full sib families with 6 equally represented clones get status number 3.4, which is "near" and which I suggest as the current tolerance limit. However, asymmetries will always appear. It is expensive and virtually impossible to avoid them, neither is it needed, as they can be compensated for by higher number so the gene diversity is still the same. It is inconvenient to do actual calculations. I suggest at least 25 clones from the progeny of at least 6 parents and hope that this compensates for the asymmetries and still results in a status number near 4 in field plantations.

Experience elsewhere

Systems of mass-multiplication of cross families by rooted cuttings have been successfully used for decades in P radiata in NZ and hoop pine in Queensland and replaced open pollinated seed orchards since a decade. Controlled crosses for seedling forestry is expanding in southern United States and comprises several percents of the seedlings, and this practice is seen so favourable as it is used in some commercial operations even without vegetative propagation.

Variants of "family-forestry", thus multiplying assumed good families mainly by rooted cuttings, is practised in Ireland and UK for Sitka spruce. The recent magnitude is about 10 million plants annually and it has been in use since more than a decade. Even with Swedish Norway spruce where exists forestry based on controlled crosses, but not at a large scale (100 000 plants/annually), which in itself is somewhat discouraging (if it was profitable "the market forces" would expand the use).

Web (functioned early 2009):
http://www-genfys.slu.se/staff/dagl/Meetings/Finland08/VegPropFinland08.htm

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Working Papers of the Finnish Forest Research Institute 114: 16–19

Clones in Finnish tree breeding

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Most tree breeding programmes utilize testing systems based on seedling stock to assess the additive genetic value of candidates and to select the best of them to breeding and seed production populations. Another alternative is to use vegetative propagules for genetic testing. Today, it is widely recognised that clones offer potential advantages over seedling testing, enabling more precise information on candidates (Fig. 1) and far more efficient genetic testing and selection in terms of space and time (Fig. 2). Furthermore, clonal propagules may be subjected to stress treatments and various other type of destructive measurements, thus enabling the breeder to obtain diverse multi-trait information on the candidates. On the other hand, it is also recognised that clone testing can lead to slightly distorted selection due to non-additive gene effects and non-genetic propagation effects (rooting problems and plagiotrophism) that may yield biased estimates of the additive breeding values. Such effects are, however, usually assumed to be small by comparison to the benefits of vegetatively reproduced test materials.

Because of the aforesaid advantages, clone testing is becoming a more important activity of the mainstream tree breeding in Finland. The breeding of hybrid aspen has already been based on the evaluation of clonal performance in field tests. Moreover, clone testing by means of stem cuttings will play the dominant role in the genetic testing of the 2nd cycle selections in Norway spruce, which will commence in the early 2010's.

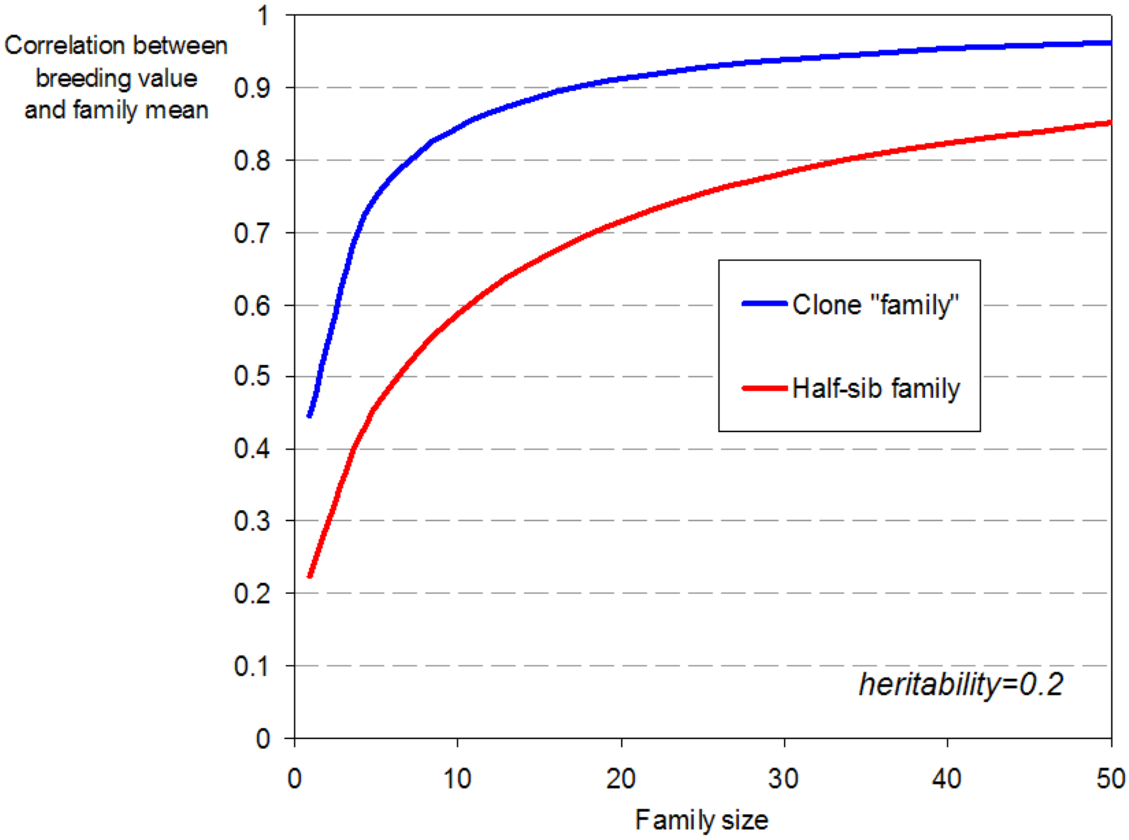

Fig. 1. The relationship between the accuracy of selection (measured as the correlation between the true breeding value and the family mean) and the family size applying either vegetative propagules or open-pollinated progenies of a candidate in genetic testing.

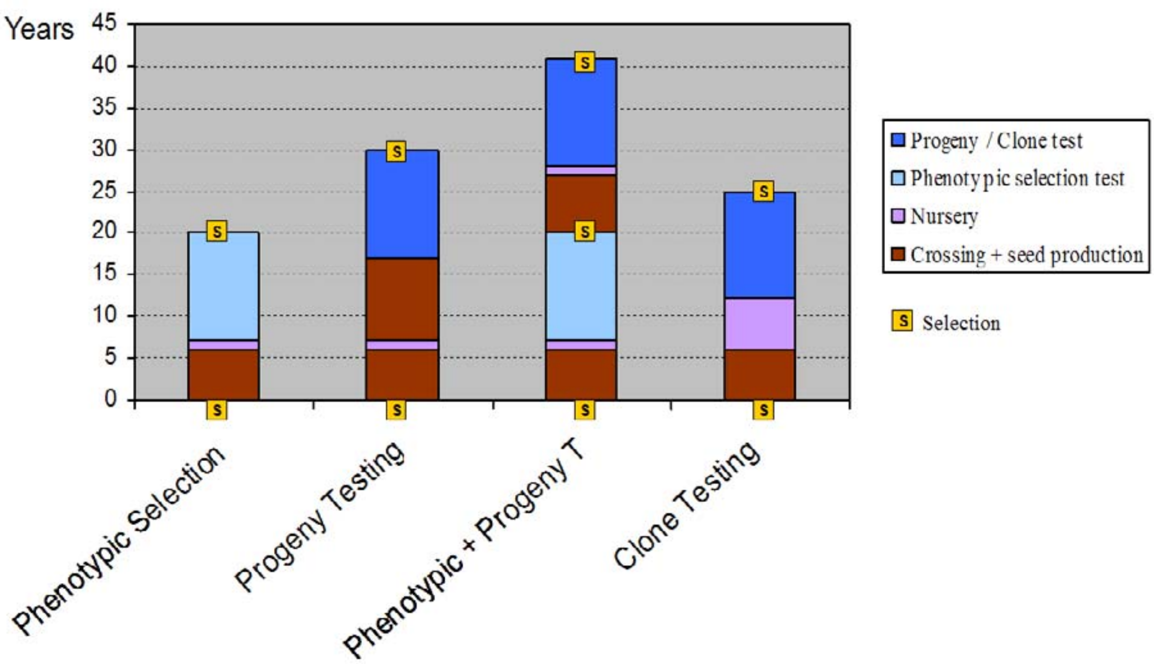

Fig. 2. Approximate lengths of breeding cycles in four alternative breeding strategies.

According to the current breeding plan for Norway spruce, the 2nd generation candidates are selected either from within full-sib families (offspring of the best tested plustrees) in a nursery-bed or in some cases, in 5 to 7 years old progeny trials which comprise open-pollinated offspring of untested plustrees. In these young progeny trials, all trees are measured for early height growth and screened for a number of traits such as branch angle, growth rhythm, frost damages, and the presence of diseases (Fig. 3). The most promising 10–15 trees within each family are selected as candidates to undergo a more careful clone testing. When candidates for clone tests are selected without the preceding visual screening in the field (i.e., in the nursery) double as many candidates are selected (30). Clone trials will be replicated in 4–6 locations, with 8 ramets per clone and per trial. The clone tests will provide the information used to form the 2nd generation breeding populations in Norway spruce in the late 2020's.

Fig. 3. Phenotypic selection of candidates in young progeny trials precedes clone testing in some breeding lines of Norway spruce. About 10–15 candidates are selected within each family. Preselection mainly focuses on vigor and traits of adaptive significance. For instance, all trees showing lammas growth (the inset photo) are rejected. (Photos: Matti Haapanen)

Table 1. The genetic testing strategies for various tree species involved in the Finnish tree breeding.

Species	Current testing strategy	Breeding cycle, years	Test material	Clone testing a viable option
Norway spruce	(Preselection +) Clone testing	25	Stem cuttings	Ongoing
Hybrid aspen	Preselection + Clone testing	20	Root cuttings / micropropagated	Ongoing
Scots pine	Preselection + Progeny testing	40	Seedlings	Yes?
Silver birch	Preselection + Progeny testing	30	Seedlings	Yes
Siberian larch	Phenotypic selection	17	Seedlings	Yes
Black alder	Phenotypic selection	20	Seedlings	Yes

Scots pine is a more difficult species to propagate vegetatively than Norway spruce. For the time being, Scots pine breeding in Finland is planned to be based on two-stage testing (preselection at age 15 followed by progeny testing) on seedling stock. Completing a breeding cycle in Scots pine is expected to take around 40 years, thus considerably longer than in Norway spruce (Table 1). Developing a workable clone testing system for Scots pine would therefore result in significant savings in time and faster realization of advanced generation tested seed orchards. A joint Nordic project to develop methods to obtain a sufficient number of rooted cuttings from young pine seedlings for clone testing is now underway. It is anticipated that clone testing could adopted for use as a supplementary testing method in several breeding lines of Scots pine in the late 2010's, provided that the current obstacles in the vegetative propagation will have been resolved by that time.

Working Papers of the Finnish Forest Research Institute 114: 20–22

Field behaviour of Scots pine cuttings compared to seedlings

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Scots pine (*Pinus sylvestris* L.) growth and stem form characteristics between cuttings and seedlings of the same origin were compared in a ten-year field trial. The seedlings were taller than the cuttings throughout the experiment, but the growth patterns - profiled by the relative growth rate - were similar in both groups. There were some changes in stem form characteristics after 9 and 10 years in the field, indicating that the use of cuttings for stem quality selection at an early age has no advantages over the use of seedlings. The consistent performance of cuttings compared to seedlings of the same origin indicates that the field performance of cuttings is a valid measure of their genetic potential and that cuttings can be used to speed up selection in breeding programmes with Scots pine.

Keywords: Scots pine (*Pinus sylvestris*), rooted cuttings, field trial

Introduction

In Finland the breeding of Scots pine started in the beginning of the 1940s and has recently reached the transition to the second breeding cycle (Haapanen and Mikola 2004). The genetic improvement of Scots pine using traditional breeding methods - selection of parents, crossings, rearing of the offspring, progeny tests, backwards selection - is a slow process which could be speed up by using vegetatively propagated material in the breeding programmes. Also, the expected genetic gain from within-family selection has been reported to be higher using cloned material instead of seedling families in *Pinus taeda* (Isik et al. 2004) and the economic input in cloning methodology pays off in shortened breeding cycle with the same or better genetic gain (Danusevičius & Lindgren 2002, Haapanen & Mikola 2004, Isik et al. 2004).

During this work our objective was to find out if the growth and the field performance of rooted Scots pine cuttings is comparable to seedlings of the same origin and can the clones be used to get a higher genetic gain from breeding tests and to speed up the breeding programs of Scots pine.

Material and methods

The Scots pine seeds for stock plants originated from two seed orchards (Varkaus and Imatra) and from one controlled cross (E104 x E1101). The 2-5 years old stock plants were sprayed with bentzyladenin solution (0.25 mM and 0.5 mM) for four weeks starting in June 1989 and in spring 1990 all shoots that had elongated 1.5 cm or more were cut, soaked in 0.5 mM IBA for 20 hrs and stuck to peat-sand-perlite mixture (40:40:20). The control seedlings were sown at the same time with the rooting of the cuttings. The field experiment was established with 2 yrs old cuttings (n=158) and seedlings (n=300) in July 1992 in Haapastensyrjä Breeding Station, Läyliäinen as a completely randomized test of single seedling/ramet plots, 1.8 x 1.8 m. The height and stem diameter at breast level were measured and the bole straightness assessed on a scale of 1-3 in 2001 and 2003. Relative growth rate, stem taper and stem volume were calculated as described by Niskanen et al. (2008). The effect of plant type (cutting or seedling) and origin (Imatra, Varkaus or E104 x E1101) and their interactions, on height, dbh, vol. and stem taper were assessed by 2-way ANOVA. The difference in bole straightness between cuttings and seedlings was analysed using Pearson Chi-Square test. All tests were performed using SPSS for Windows, release 15.0 (SPSS, Chicago, IL, USA).

Results

The cuttings were shorter than the seedlings throughout the experiment (Fig. 1). However, the origin (d.f. = 2, F = 4.611, $p = 0.010$) of the plants had more significant effect on the height than the plant type (d.f. =1, F = 5.185, $p = 0.023$) after 10 years on the field.

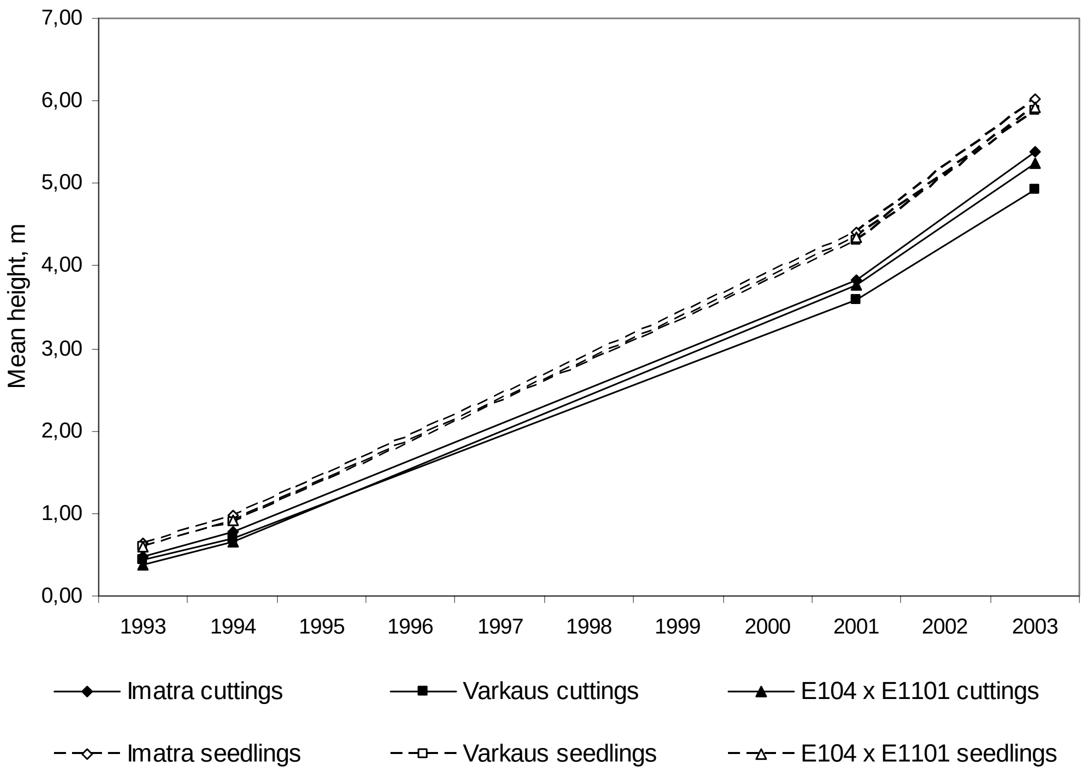

Fig. 1. Mean heights of Scots pine cuttings and seedlings of three different origins in the field trial in 1993-2003 (from Niskanen et al. 2008).

The RGR patterns of both the cuttings and the seedlings stabilized before the 9th year in the field, indicating similar growth patterns of the both plant types (Fig. 2). Also, there were no differences in dbh (d.f. = 1, F = 1.772, p = 0.190), stem volume (d.f. = 1, F = 2.165, p = 0.174), stem taper (d.f. = 1, F = 0.688, p = 0.408) or bole straightness (d.f. = 2, X^2 = 0.853, p = 0.653) between the cuttings and the seedlings at the end of the field trial.

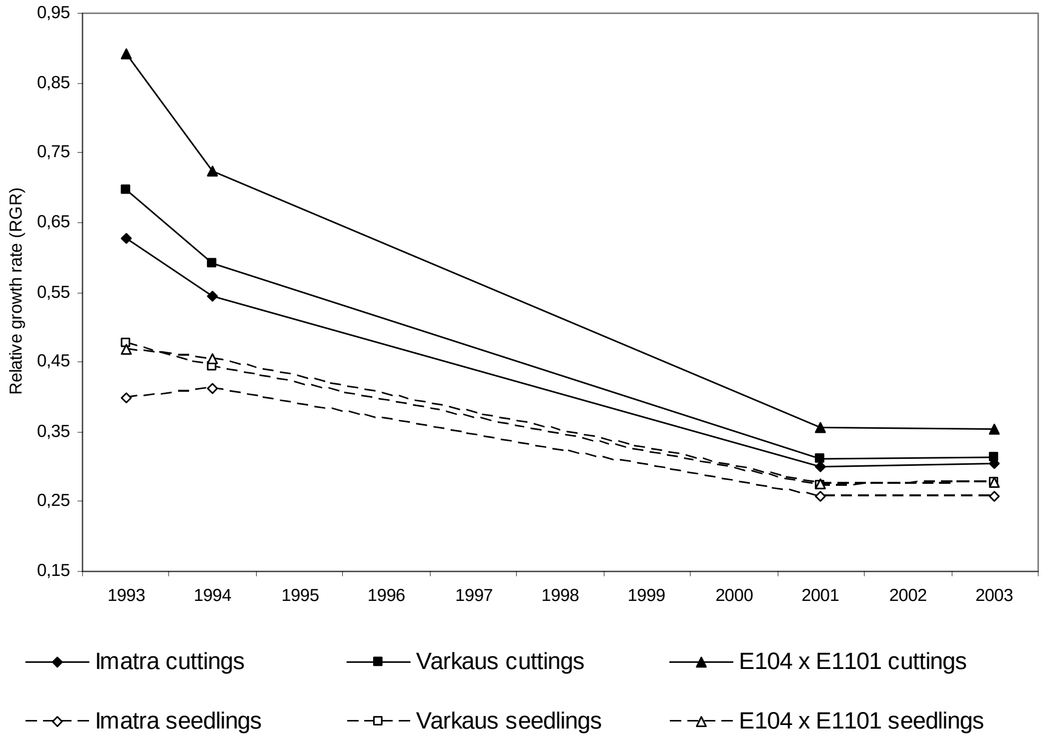

Fig. 2. Scots pine relative growth rates in a field trial in 1993 - 2003 (from Niskanen et al. 2008).

Conclusions

The results of this experiment was that the Scots pine seedlings were taller than rooted cuttings of the same origin indicating that the height growth of Scots pine seedlings is faster than the height growth of rooted cuttings. However, the other observed growth parameters were similar in both plant types indicating similar growth patterns and the effect of plant origin to height was more significant than the effect of plant type. Therefore, we conclude that the height growth of Scots pine families may be predicted using cloned material and that rooted cuttings may be used to speed up Scots pine breeding programmes.

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Working Papers of the Finnish Forest Research Institute 114: 23–26

Conifers in landscaping – potentials and problems

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Exotic conifer species are used in urban landscaping to enrich the limited number of native local taxa available. Many of these widespread species, forms and varieties have been selected for central European conditions and are thus well adapted to more favourable environments. Often the use of southern exotic origins leads to premature renewal of conifer plantations in the Finnish climate. Indigenous conifer species, cultivars and forms and local seed sources of exotic conifer species should thus be selected and tested for hardiness in Nordic urban environments and landscaping.

Keywords: native, exotic, conifer species, cultivar, urban environment, landscaping, selection program

Introduction

Many exotic conifer species and their various origins are already planted in forestry collections and stands of the Finnish Forest Research Institute and Mustila Arboretum, Elimäki (Silander & al. 2000). Interesting selection material could also be found from other collections, plantations, smaller arboreta or even from city parks. Most of the forms of native conifer species have been found and recorded in registers in early stage of forest tree selection work (Oskarsson & Nikkanen 1999). Many exotic forestry seed sources and native conifer forms could directly, without any further selection, be used for landscaping purposes (Silander & al. 2000, Oskarsson & Nikkanen 2001).

Sæbo & al. (2005) made a review of procedures in urban tree selection. Only limited attention has been paid to conifers. An ideal conifer tree species should have good overall adaptation to local climate and have reasonable tolerance to urban environments and stresses. Finally a tested conifer accession should also show a large phenotypic plasticity over a range of different conditions. Pragmatic selection programs could be the cheapest mean of utilising plant materials already in plantations and production (Sæbo & al. 2005).

Reduced size, slow growth habit combined with attractive appearance – traits for urban conifers

Many of the forms and varieties of Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), common juniper (*Juniperus communis*) and common yew (*Taxus baccata*) are already well described in the literature. (Gelderen & Hoye Smith1997, Hillier & Coombes 2007, Krüssmann 1983). There are only limited statistics available on the use of conifer taxa in urban green areas in Finland. Customer based information of the most desired forms of conifers and their traits would also be valuable for future selection work.

Several of these forms of conifers recently found from nature had their early parallels in plant nurseries already in the 19th century. Some of these old garden varieties are even today in nursery production or planted in city parks. One of the oldest conifer cultivars is *Picea abies* 'Clanbrassiliana'. It was originally discovered in Northern Ireland on Lord Clanbrasil's Estate around 1790. It is a very slow growing bush. In the dormant season it is conspicuous by its virtue of brown winter buds and small branchlets that vary in vigour (Hillier & Coombes 2007). Many spruce forms, for example, nearly similar to this description could still be awaiting discovery in our forests.

Fig. 1. Cultivars of Norway spruce (*Picea abies*), common juniper (*Juniperus communis*) and thuja (*Thuja occidentalis*) with native Scots pines (*Pinus sylvestris*) in a conifer plantation of Sapokka Park in Kotka, Southern Finland. (Photo: Juha Raisio 28.8.2008)

In the future private and public urban gardens will tend to be smaller, this is one of the main tendencies in urban landscaping. The most obvious need will be for dwarf conifers as well as semi high forms and cultivars (figs. 1 and 2). A restrained habit resulting from genetic modification of many conifers will also be of great value also in city parks. Slow growth and reduced size combined with special appearance, for example a columnar form, in many cases makes for less pruning and saved resources in later maintenance work. In these challenging days of climate change, the need to broaden the genetic base of planted tree and shrub taxa in urban environments should also be kept in mind.

Fig. 2. Columnar and carpet forming cultivars of common juniper (*Juniperus communis*) in Sapokka, Kotka. (Photo: Juha Raisio)

New conifer cultivars for urban use – an initiative of a special selection program has been taken

Central European plant nurseries have climatic advantages and longer traditions in conifer plant production than their Finnish counterparts. Much of the conifer plant material used in urban areas in Finland is originates from abroad. A number of well tested hardy conifer forms and varieties could be beneficial for local production. For example, the Swedish ‘E-planta -system‘ of broadleaved trees and shrubs has been some kind of success story for the Swedish plant nursery business.

The initiative for a special Finnish program for evaluating and selecting hardy conifer material for urban use has already been taken. The program will continue by selecting a limited number of new conifer forms and cultivars for immediate nursery propagation and some (4-5) new urban conifer arboreta should also be established. A broad number of conifer taxa planted in different urban environments could serve as long term base for future selection and breeding efforts made in co-operation with Finnish Forest Research Institute, tree nurseries and participating cities.

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Working Papers of the Finnish Forest Research Institute 114: 27–30

Hardy options for landscaping – domesticated exotics and special forms of Nordic conifers

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Tree nursery operators and landscape architects are interested in domesticated exotics and special tree forms of native conifers. However, the demand and supply of hardy, domesticated exotics and special tree forms have not always been balanced. In Finland, as in the entire Nordic region, the market demands consistent and sustainable production of hardy, decorative ornamental conifers. These could replace the less hardy imports from Central Europe and would be suitable for use in landscaping under the harsh Nordic conditions. A large number of experimental plantations with exotic conifers were established in the 1920's and 1930's in different parts of the country. The aim of the species trials was to find new tree species for the forestry. However, only some of the exotic species have potential as economically important timber species. Instead, many species have become important in landscape management and ornamental use. Among the normal trees in our forests there are, as rare whims of nature, a variety of peculiar tree forms. In its forest genetic register, Metla has records of 1300 individual trees that are genetic deviants of our native tree species. Many of these have also been conserved in clone archives and arboretums. The utilisation of special tree forms usually requires vegetative propagation. In this way, the traits of the parent tree are passed as such on to the cloned offspring.

Keywords: decorative trees, exotic conifers, landscaping, mutation, special tree forms, vegetative propagation

Domesticated exotic conifers

In Finland, as also in Sweden and Norway, only four native conifer tree species can be found: Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), Common juniper (*Juniperus communis*) and Common yew (*Taxus baccata*). However, more than 50 conifer species from different parts of the world can be grown successfully in our conditions.

In the 19th century a number of *Larix sibirica*, *L. decidua*, *Abies sibirica* and *Pinus cembra* plantations were establish in Southern Finland (Fig. 1). Systematic trials with exotic conifers started in the early 20th century at Mustila Arboretum, and a little later at the Finnish Forest Research Institute (Metla). In the 1920's and 1930's, a large number of experimental plantations with exotic conifers were established in several research areas located in different parts of the country. The aim of the species trials was to find new tree species for the forestry. However, only some of the exotic species have potential as economically important timber species. Instead, many species have become important in landscape management.

By the end of the 1990's the experimental plantations had reached the age of almost one tree generation. In 1996-97 an inventory on the performance of 73 exotic conifer taxa (species, subspecies and varieties) from 10 genera was carried out. A total of 605 tree species plantations, covering an area of 300 hectares, were evaluated. Good performance, i.e. good resistance against different forms of damage, and growth similar to that of the species in its natural habitat, was estimated for 20 taxa. Fair performance, i.e. rather good survival, but growth lower than that in the natural habitat, for 20 taxa, too. From these about 20 species can be recommended for landscape management and ornamental use in Southern Finland, and somewhat smaller amount of species in more northern parts of the country.

Most of the tree species plantations estimated as good or fair performance can also act as seed sources. When a new generation of tree species plantations was established, the seed was collected mainly from these plantations. During the years 1994-2006 a total of 60 conifer taxa in an area of 100 hectares were planted.

Fig. 1. A Cembra pine (*Pinus cembra*) plantation at Punkaharju.

Special forms of native forest trees

Among the normal trees in our forests there are, as rare whims of nature, a variety of peculiar tree forms: weeping spruces and pyramid pines, dense dwarf spruces, witches' broom pines, golden spruces and pines, as well as red and golden birches, cut-leaf birches and alders (Fig. 2). In its forest genetic register, Metla has records of 1300 individual trees that are genetic deviants of our native trees. Many of these have also been conserved in clone banks and arboretums.

Special forms of trees found in the nature are caused by a mutation, i.e. a change in its genetic material. If the change has already occurred in pollen or an egg cell, the altered genetic code will be passed on to all cells of the new individual. Example of such mutation is golden spruce. If the mutation occurred in the vegetative bud, the change will be limited to that part of the tree, which is developed from the genetically altered bud. The witches' brooms, found in spruce and pine are considered to be results of bud mutations.

The most common way to use special tree forms is as ornamental trees. The utilization of special forms usually requires vegetative propagation. In this way, the traits of the parent tree are passed on to the cloned offspring. Vegetative propagation includes the use of grafts, cuttings and tissue culture.

Fig. 2. Special forms of native forest trees: weeping spruce (A), narrow-crowned golden spruce (B), dense dwarf spruce (C) and golden pine (D).

By making artificial crosses between rare forms of trees a breeder can create new combinations of traits that have not been found anywhere in the nature. Recently, a small number of crossings between various forms of forest trees have been produced in order to find new, decorative tree forms for ornamental use. New forms raised this way are crossings between the red coloured spruce (*Picea abies* f. *cruenta*), the weeping spruce (*P. abies* f. *pendula*) and the compact globe spruce (*P. abies* f. *globosa*). A new form of desired characteristics can be multiplied into millions of copies by means of vegetative propagation.

Hardy options for landscaping

Although broad-leaved trees are without leaves and not green more than half of the year even in the southern most part of Finland, conifer trees are very seldom used as ornamental trees in landscaping. When ornamental conifers have been used, only very few species have been used, and the planting material has usually been imported from Central Europe. In many cases the material has not been hardy enough even in Southern Finland.

Tree nursery operators and landscape architects have been interested in domesticated exotics and special tree forms of native conifers for several years. However, the demand and supply of hardy, domesticated exotics and special tree forms have not always been balanced. In Finland, as in the entire Nordic region, the market now demands consistent and sustainable production of at least a small selection of hardy, decorative ornamental conifers. These could replace the less hardy imports from Central Europe and would be suitable for use in landscaping under the harsh Nordic conditions (Fig. 3).

Fig. 3. Weeping spruces at University Campus in Jyväskylä.
(All photos by Teijo Nikkanen)

Working Papers of the Finnish Forest Research Institute 114: 31–33

Investigation of conifers mutation forms in Latvia and the possibilities in selecting new sorts

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Kalsnava arboretum and nursery

Kalsnavas arboretum is established on the 18th of September, 1975. The whole area of the arboretum is 143.96 hectares. Plantations of mutation forms are settled down in the part of arboretum where the low forms collections are. Gathering and selecting conifers mutation forms in Latvia was started in 1982. This work Kalsnavas arboretum did together with National botanical garden. These collections are duplicated at Kalsnavas arboretum and at National botanical garden to ensure that they wouldn't be withered away. On the whole, till the year 1995 there were gathered more than 50 different conifers (Norway spruce, Scots pine) mutation forms. These forms have been multiplied by grafting and rooting, and by sowing seeds of these forms.

Till 2004, the arboretum kept in order and supplemented the collection, and increased the amount of plants and sold them. In 2005, the arboretum was divided in two parts – Kalsnava arboretum and the nursery 'Kalsnava'. Kalsnavas arboretum continues the supplementing of collection and gathering mutation forms, and conifers selecting work. Nursery 'Kalsnava' increases the amount of ornamental plants, relying on the base of Kalsnavas arboretum collection, and sells the plants. From 2006, they started to produce hybrid aspens in larger amount by using tissue cultured crops which are used as forest planting material.

Collections of ornamental trees in Kalsnava

Kalsnavas arboretum has one of the largest collections of ornamental trees and shrubs in Latvia. There are registered 2490 taxa in the spring 2008. In arboretum territory grow more 360 different forms and varieties of conifers, including 107 different Norway spruce and 54 Scots pine forms and varieties. The most valuable of local origin mutation forms are *Pinus peuce* ‘Latgale’ (figure 1), *Picea abies* ‘Dundanga’ (figure 2), *Pinus sylvestris* ‘Earl nest’, *Pinus mugo* ‘Dwarf’, *Abies sibirica* ‘Nana’ and *Abies arizonica* ‘Dwarf’.

Fig. 1. *Pinus peuce* ‘Latgale’ in Arboretum Kalsnava (Photo: Janis Zilins)

Fig. 2. *Picea abies* (Dundanga) in National botanical garden. (Photo: Andrejs Svilans)

Raising new ornamental conifer varieties

Till 2005, there were 36 different sorts of mutation form collection. Beginning in 2005, with the help of National botanical garden, Kalsnavas arboretum started gathering, estimating and selecting of conifers mutation forms. In 2005, it has been gathered 36 mutation forms, in 2006 51 (32 pine and 23 fir trees). Each sort of mutation forms is increased witch grafting method in 10 copies. Now there are being made criterions for making tests for distinctness, uniformity and stability and for registration of new conifers forms. From gathered conifer mutation forms in spring of 2009 will be made a collection of varieties comparing to estimate potential conifer varieties.

Fig. 3. Ornamental plants in Kalsnava nursery. (Photo: Janis Zilins)

Fig. 4. *Picea abies* cuttings in Kalsnava nursery. (Photo: Janis Zilins)

From the arboretum collection of conifers varieties, Nursery 'Kalsnava' increases 96 taxa with grafting method and 131 taxa with rooting methods (figure 3). In the aggregate nursery 'Kalsnava' for one year realization makes 89000 conifer cuttings (figure 4), 23000 grafts (figure 5) and 20000 seedlings (figure 6) and so there are produced 132000 new plants.

Fig. 5. One year old mutation form grafts of *Pinus sylvestris*. (Photos: Janis Zilins)

Fig. 6. Seedlings of mutation forms. (Photos: Janis Zilins)

Working Papers of the Finnish Forest Research Institute 114: 34–38

Chip-budding and apical grafting – breeders' tools

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Traditional grafting techniques for multiplying trees to seed orchards

One of the problems facing forest-tree breeders is the long time it takes to complete a breeding cycle, and the almost-as-long time before the best trees can be mass propagated, and the improved trees put into practical use in forestry.

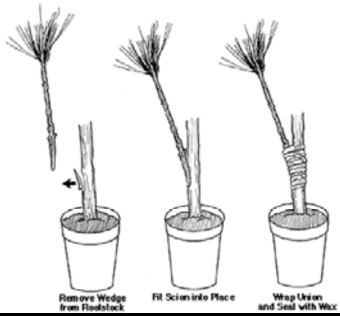
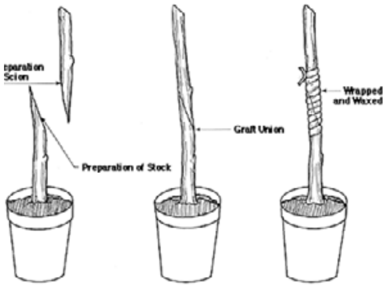
In time, somatic embryogenesis and tissue culture will no doubt be used to shorten the rotation period for both testing and mass propagation, but such methods are far from fully developed in respect of our conifers. If we want to produce copies of a tested and selected tree to produce seed in seed orchards, the only method available today for propagation of Scots pine and Norway spruce older than ten years is grafting. The commonly used techniques include side grafting and apical i.e. whip or slice grafting (Table 1).

It takes a long time to produce 300-400 suitable scions for mass propagation from a tree. Even if we use secondary propagation to produce scions, it will still be at least fifteen years from selection to mass propagation in seed orchards.

Chip-budding as an alternative grafting method

With a view to reducing the waiting time between selection and mass propagation, in 2003 we initiated a project that focused on the possibility of propagate pine using the adventitious buds located between the needles. Theoretically, this should provide sufficient stock for the mass production of 300-400 ramets from very young trees. As regards spruce, we looked at the feasibility of using all the buds on a spruce shoot for grafting - rather than simply grafting a single shoot. In addition, for both pine and spruce, we would further develop the technique of apical grafting of thin scions onto small rootstocks. (This project was financed by the Forest Tree Breeding Association.)

Table 1. Comparison of side grafting and apical grafting techniques.

Property	Side graft	Splice graft
		
Root stock	+ independent of diameter	- equal diameters needed
Scions for grafting	+ more suitable scions	- fewer suitable scions
Grafting technique	- difficult, labour intensive	+ simple, efficient
Management	- require repeated pruning	+ require pruning 1-2 times
Growth	- slow, minimum 2 years to delivery size	+ high, delivery possible in 1 years
Morphology	- branch like growth	+ straight growth

The grafting of adventitious spruce and pine buds was done using the same principle as that for grafting of roses; namely, that a cut is made below the bud to form a bud chip of wood that is then fixed to the top of a rootstock, in which a matching cut of the same size has been made, i.e. chip budding. To activate the dormant adventitious bud on the stock, all the buds on the stock are removed.

In the case of pine, the method worked with only a few clones, and only after repeated pruning of the stock to break the dormancy in the grafted adventitious bud. To break dormancy in the adventitious bud we investigated treatments applied to the mother tree one year before grafting. By cutting back the shoots on the mother tree, buds developed between the needles on the tops of the cut shoots (fig. 1a-b). These buds flushed immediately after grafting (fig. 1c-d), and it appears that the method, in principle, works on all the clones. As regards pine, however, considerable development work will be necessary on the production of suitable buds, and on the methodology of chip-budding, before the method can be used in operational mass propagation. The comparison of the estimated costs in the different grafting methods is presented in the Table 2.

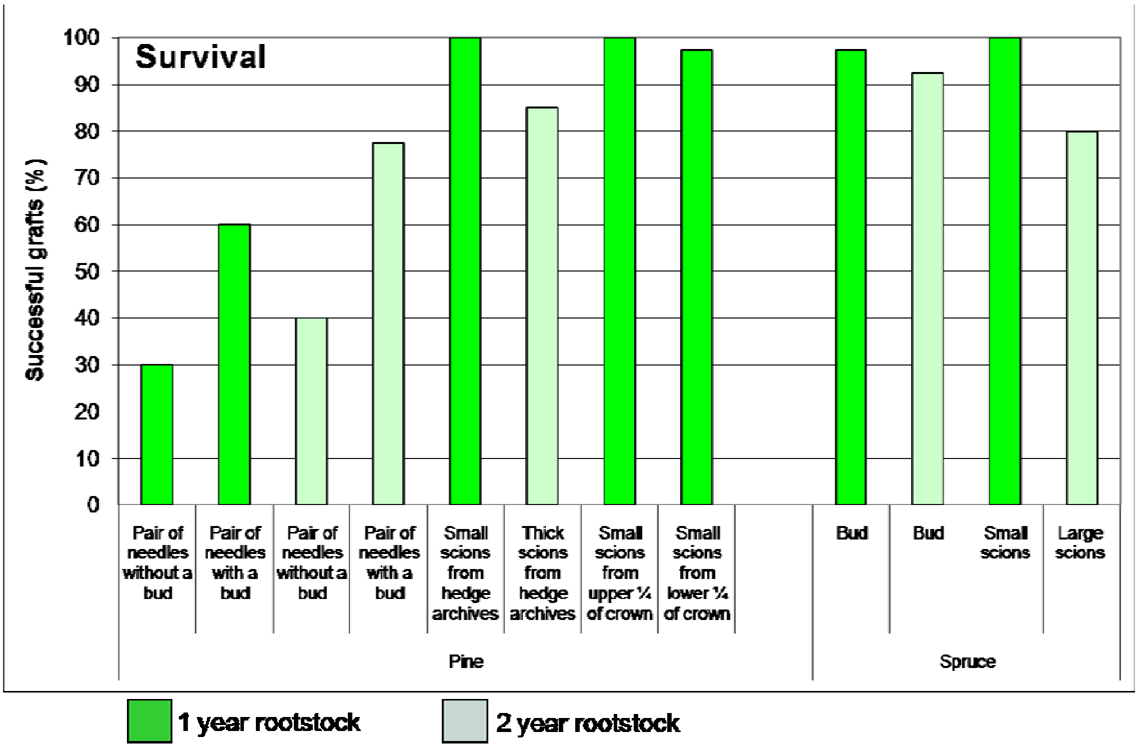
Fig. 1. Chip-budding in Scots pine. **A**) In untreated mother plant adventitious buds are hidden between the needle pairs, but **B**) they develop following pruning treatment of the mother plant. **C**) An adventitious Scots pine bud grafted on the rootstock, being **D**) developed into a nice graft after only one growing season. (Photos: Jörgen Hajek)

As regards spruce, chip-budding worked so well that the method, after fine-tuning of the work and timing, can be used in mass propagation on an operational scale of grafts for seed orchards (fig 2). Comparison of different grafting methods in pine and spruce is shown in figure 3.

Fig. 2. Chip-budding in Norway spruce. **A**) In the spruce mother plants, there are plenty of buds available for **B**) chip-budding on the rootstock. (Photos: Jörgen Hajek)

Table 2. Estimation of costs in SEK for different grafting methods. Condition: grow 5000 grafts for delivery.

Activity	Apical grafting 2 year old rootstock in 3 litre container Delivery 2 year after grafting Height 60 cm	Apical grafting 1 year old rootstock in 1 litre container Delivery 1 year after grafting Height 20 cm	Chip-budding 1 year old rootstock in 3 litre container Delivery 1 year after grafting
Pruning of mother tree			2,60
Collection of scions (or buds)	2,10	1,80	2,40
Production of rootstock	19,00	13,10	13,10
Transplantation of rootstock	5,00	3,80	8,80
Grafting	15,40	17,50	34,50
Labeling	3,75	3,75	3,75
Cultivation of grafts	63,00	24,50	44,60
Pruning of rootstock	3,20	5,20	20,70
Administration	4,20	3,80	5,40
Delivery	3,30	2,60	3,30
Total cost	119,00	76,00	139,00



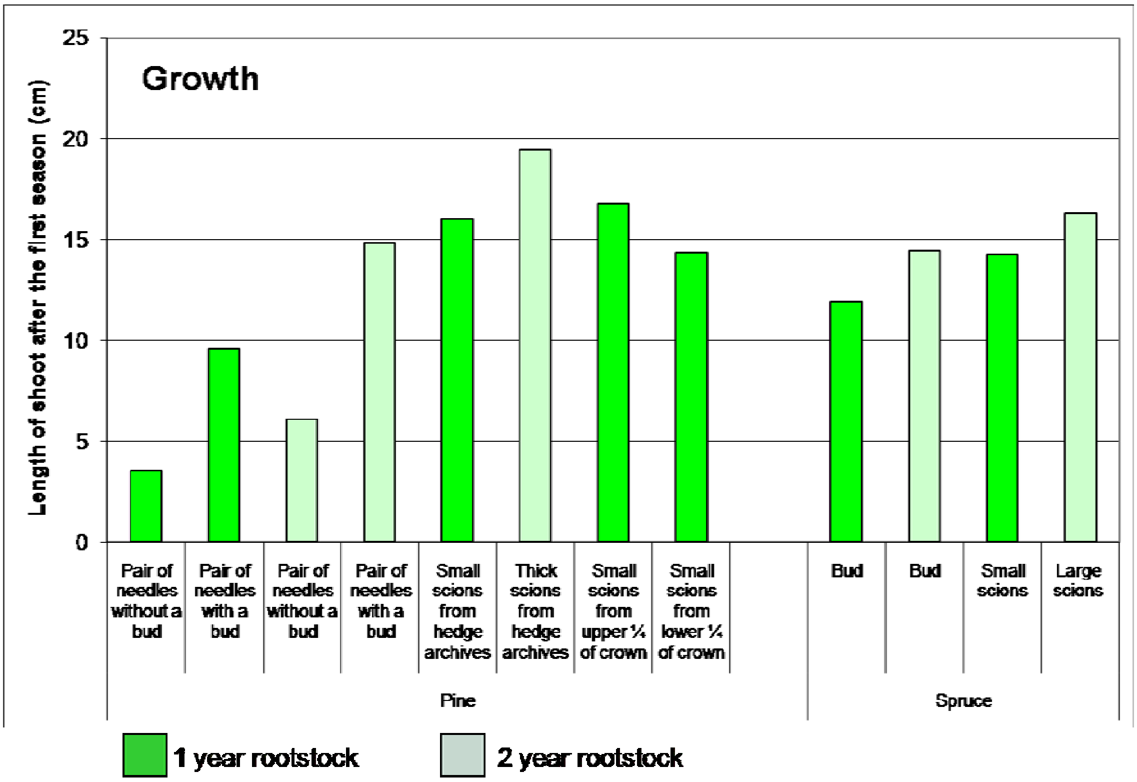


Fig. 3. Comparison of the grafting results using different methods in Scots pine and Norway spruce.

Working Papers of the Finnish Forest Research Institute 114: 39–43

Successes and failures in forest tree cutting production in Finland

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This article is a review on the development work and experiences of vegetative propagation of forest trees in Finland. Some attempts and achievements in Scandinavian countries and the other world are also shortly commented.

Keywords: vegetative propagation, cuttings, rooting, clonal forestry

Early attempts in Finland

In Finland, experiments with rooting of cuttings were started by the Foundation for Forest Tree Breeding (FFTB) in 1962, with facilities donated by the Finnish Plywood Industry. Many species were included from the beginning, e.g. birches, Norway spruce, Scots, pine, larches, hybrid aspen, alders, maple and elms (Fig. 1). The first results were very promising, leading to the conclusion that "it seems possible that cutting propagation will soon become a shortcut to the application of tree breeding results in practical reforestation" (FFTB 1962).

Fig. 1. Examples of cuttings rooted in early 1960's. From left: birch, alder, juniper, Scots pine and Norway spruce. (Photo: FFTB)

In the 1960's the attempts were focused on a single clone, the famous triploid curly birch "Olli-Visa" (*Betula pendula* v. *carelica*). However, the success of rooting varied greatly from year to year with this clone, and also with other broadleaved species and Scots pine and larches as well, and high mortality after transplanting outdoors and especially during the first winter proved to be big problems. With broadleaved species, best rooting results were generally achieved with sturdy current year's shoots forced in potted greenhouse plants in early spring. The general experience was that almost everything could be rooted (including e.g. roses and apple trees), but induced roots in summer cuttings were very tender. Already transplanting outdoors caused much, mortality, and most plants died during the next winter.

Gradually the interest was shifted to conifers, Norway spruce and Scots pine, with which previous year's resting shoots and early spring rooting in mist-irrigated greenhouses proved to much better than current year's soft shoots. In 1969 average rooting in both species was 75 %, and with some clones up to 100 %. FFTB optimistically reported: "Rooting of spruce cutting is already so well governed that a shift from experimental scale to practical mass production scale can begin" (FFTB 1970).

Rooting experiments were still continued through the 1970's with many species, including birches, alders, hybrid aspen, *Pinus contorta*, larches, in addition to Norway spruce. In 1977, 80-95 % rooting was achieved with all mentioned species, but further raising remained problematic. Scots pine was dropped out in 1972, because of low availability of scions and poor growth form of the produced cutting plants (FFTB 1977).

Attempts shifted to Norway spruce

In the late 1970's the attention in FFTB was completely focused to Norway spruce, following the promising examples from Germany and Scandinavian countries (e.g. Kleinschmidt 1973, Roulund 1971). Through the 1970's, FFTB raised 150 000 to 300 000 spruce cuttings annually (Fig. 2). Clone testing was started, with some 2000 clones up to 1982. A few attempts were made for the mass production of spruce cuttings, but the results were not very promising because of plant quality problems, mainly due to the physiological ageing of clones (FFTB 1983).

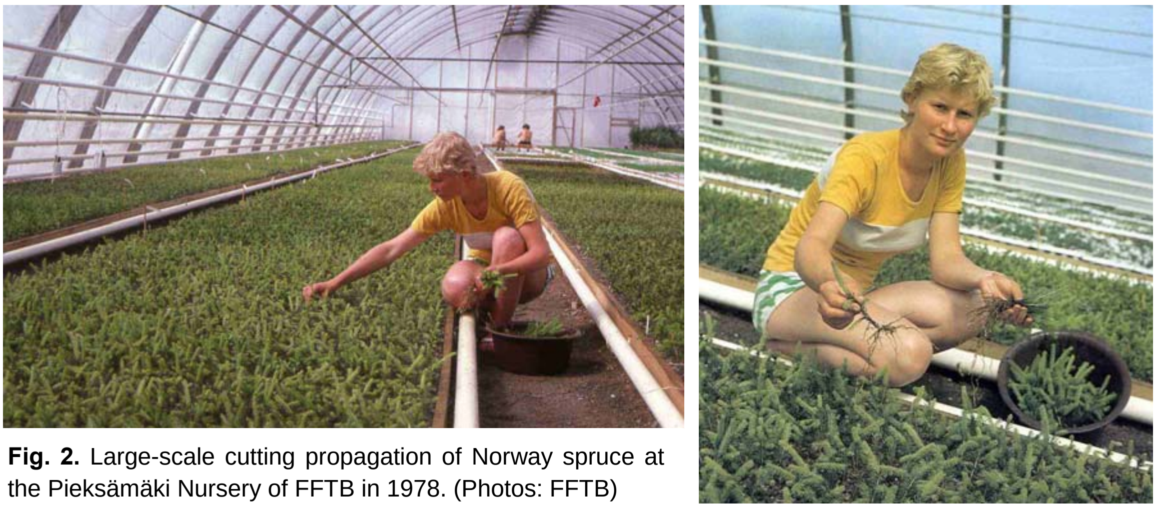

Fig. 2. Large-scale cutting propagation of Norway spruce at the Pieksämäki Nursery of FFTB in 1978. (Photos: FFTB)

Clonal tests showed up to 50 % superiorities in early height growth for the best clones, as compared to the average of clones and to seedling standards. Many of the best clones in the tests of FFTB proved to be provenance hybrids (Finland x Central Europe) (FFTB 1982, 1987) (Fig. 3). However, physiological ageing appeared to be an almost absolute obstacle for further utilization of best clones, although the physiologically juvenile period of clones could be prolonged to some extent by hedging or serial propagation. Even slight ageing caused problems in cutting production; leaned or crooked cuttings had to be graded out of commercial reforestation plant lots, because of the quality regulations, and this naturally increased the production costs of acceptable cuttings.

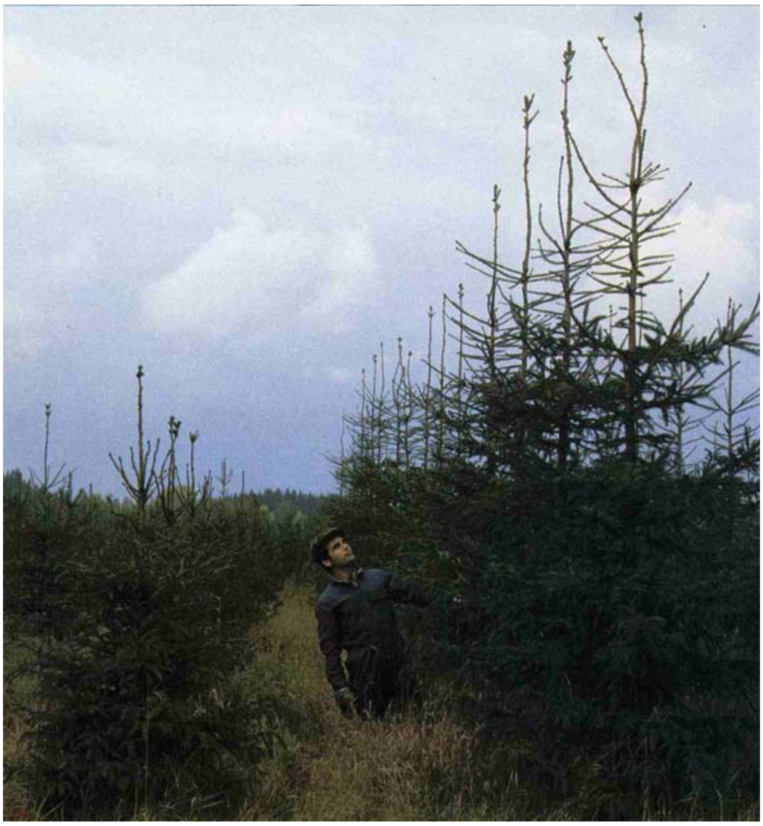

Fig. 3. A demonstration plot in Nurmijärvi, S. Finland. The single clone V 383 on the right was selected from a provenance hybrid family (Central Finland x Schielbach, Germany). At the age of 8 years from planting it surpasses the seedlings of the same family (on the left) by 78 % in height growth. (Photo:FFTB)

Experiments for the development of spruce cutting techniques were continued at a smaller scale through the 1980's. A few clonal mixtures were formed and registered, but they never reached any mass-scale production. In the 1990's interest has been shifted to bulk propagation of tested full-sib families (FFTB 1987, 1990). No commercial production has been started yet, but it may soon become a realistic alternative to seedling production, if seed production in seed orchards proves to be seriously limited by insect and disease problems. The conventional cutting propagation technique, at its present state-of-the-art, seems to be quite feasible for bulk propagation of seedling materials of Norway spruce, e.g. progeny-tested full sib families. With very young donor plants (3-6 years) rooting percentage is close to 100, and the growth form of cutting plants is quite comparable to seedlings. The price of cuttings will inevitably be higher than that of seedlings, however, because more human handwork is needed in the production of cuttings.

Vegetative propagation programs with traditional techniques of rooted cuttings have been carried out in many countries, with better success than in Finland. In Lower Saxony, Germany, some 1 million cutting were produced for reforestation in 1970's and 1980's, at a 1,3 -fold price as compared to seedlings. Obviously this program does not continue at the same level any more. Hilleshög company in Sweden produced a few million Norway spruce cuttings in some years in late 1970's and 1980's, also at a price of about 1,3 x seedling price. Hillsehög's activity was slowed down obviously because of increasing production costs, due to the ageing of clones and extra costs caused by new regulations for the mixing of clones in commercial production. There have been several cooperative Norway spruce cutting programs in Sweden: the South Swedish program in late 1970's (together with Hilleshög), the Central Swedish program in 1980-1992, and the New Central Swedish program in 2000-2004. All these programs were slowed down obviously because of the high production costs of cuttings, and resulting low demand in practical forestry (e.g. Högberg et al.1995, Lindgren 2006).

The onset of micropropagation

Quite new ideas for the clonal propagation of reforestation material emerged in the 1980's. Tissue culture method through organogenesis was first developed for birch in the University of Helsinki. Laboratory-based "micropropagation" was soon adopted by some Finnish companies for mass multiplication of birch clones (FFTB 1991). Commercial production lasted only a few years, however. The production costs were high, and the market of cloned plants remained small in practical forestry. The low demand on the forest owner's side was maybe mainly due to the lacking information of genetic gains attained through clonal selection.

Micropropagation of conifers was intensively studied in FFRI, Forest Research Institute and some universities and companies in late 1980's and early 1990's. Clonal plantlets were attained in many cases from very young seedling materials (FFTB 1985-1991). Micropropagation may be applied for the production of cloned material for research purposes, but the methods proved to be quite too slow and tedious for any practical applications.

In late 1980's the method of inducing and rooting of fascicular shoots in Scots pine was studied in the FFRI. Induction of fascicular buds with hormone treatments was successful in young and old trees, but the rooting of induced dwarf shoots was very variable. Physiological ageing proved to be decisive also with this method; generally only dwarf shoots induced on the topshoots of quite young seedling materials could be rooted (FFTB 1985-1990). The work was slowed down in the 1990's.

In late 1980's Finnish paper industry became highly interested in hybrid aspen. It was considered as a very good raw material for fine printing papers. Selection and testing of aspen clones from 20-30 years old plantations was started, clonal propagation methods through tissue culture were developed, large-scale commercial production of micropropagated plants was initiated, and the establishment of aspen plantations in practical forestry was promoted (FFTB 1995-1998). However, again the forest owners' interest remained low, maybe mainly due to the high cost of clonal plants. Therefore a cheaper, more traditional method of aspen cloning was developed, based on the rooting of root pieces in greenhouse conditions. Anyhow, the use of cloned aspen has remained at a very low level in practical forestry.

Concluding remarks

Somatic embryogenesis together with cryopreservation gives great promises for commercial production of tested Norway spruce clones in the future. This is the main current interest area in the study and development of vegetative propagation of forest trees in Finland, as well as in many other countries (FFTB 1993-1994, Lindgren 2006). There are no practical applications yet, but most optimistic guesses suggest that "artificial seed" may become even cheaper than seed orchard seed. In the longer run, tree breeders hope somatic embryogenesis to open the way to physiological rejuvenation and efficient clonal propagation of older trees tested or selected in progeny tests.

There are still some success stories going on with conventional cutting propagation and clonal forestry of spruce species. Some 1500 ha are planted annually with *Picea mariana* in Eastern Canada, and some 2500 ha with *Picea sitchensis* in Ireland and Scotland (e.g. Lindgren 2006). And of course, in other parts of the world clonal forestry has become quite well-established with some species (e.g. *Eucalyptus* spp., *Acacia* sp., *Cupressus* sp., *Pinus* sp.) and with some species it even has a very long tradition (e.g. *Cryptomeria* in Japan, poplars and willows in Europe).

At present, only a few clones of curly birch and some ornamental forms of forest trees are vegetatively propagated in a commercial scale in Finland, for special wood production and landscaping purposes.

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Working Papers of the Finnish Forest Research Institute 114: 44–47

Joint Scots pine cutting propagation project Finland-Latvia-Sweden

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A research project on Scots pine cutting propagation has been launched as a joint effort involving researchers from Finland, Latvia and Sweden. The aim is to develop pine cutting propagation to a standard that makes it possible to use for cloning candidates in the breeding programme. The key problem in this research is how to optimize the two conflicting factors corresponding to donor plant age: shoot production and high rooting success.

Keywords: adventitious shoots, donor plant, shoot production, rooting environment

Background

Vegetative propagation is a tool for speeding up the breeding cycle by enabling candidates in the next generation to be tested as clones and not by their progenies. In the Swedish spruce (*Picea abies*) breeding programme, vegetative propagation by cuttings is routinely used. The second conifer species of great economic importance in Nordic countries, Scots pine (*Pinus sylvestris*), is more difficult to propagate by vegetative means (Boeijink & van Broekhuizen 1974, Strömquist 1979, Lindgren et al. 1992). However, more recent experiments suggest that it could be possible to develop cutting propagation of Scots pine to a level that allows use in breeding. Högberg (2005) reported 54 % rooting of cuttings taken after top pruning the second year. This rooting experiment was conducted in late summer with donor plants from 46 open-pollinated families. Some experiments on smaller materials, where the rooting took place during winter, have resulted in even higher rooting success (Skogforsk, unpublished data). However, the results are not consistent and more stable results are needed to include cutting propagation as a routine operation in Scots pine tree breeding.

Rooting ability of cuttings decreases with the age of the donor plants (Boeijink & van Broekhuizen 1974, Strömquist 1979). Already a two-year old donor plant tends to give low rooting percentages, but as indicated by Högberg (2005), this is not always the case. The aging effects highlight the conflict that has to be handled in development of cutting propagation method. An old donor plant can produce more shoots suitable for cutting propagation but the rooting ability decreases. The now initiated project, involving the institutes Metla in Finland, Silava in Latvia and Skogforsk in Sweden, aims at finding the best solution of this unfavourable relation.

Research approach

The present model for cutting propagation starts with sowing of in early spring and then cultivation with a generous fertilisation program to get large-sized plants with well-developed root systems. In spring the following year, the donor plants are top pruned just below the point where the first year's bud was formed. Adventitious shoots from needle-pairs develop during the summer year 2 and is harvested and rooted in winter year 3. Fig. 1 below shows the sequence of events. Observe that with sowing in early spring a second flush the first year can be expected like the donor plant in A in Fig. 1.

Fig. 1. Cutting propagation of Scots pine: **(A)** donor plant ready to be pruned, **(B)** pruned donor plant with developing adventitious shoots, **(C)** adventitious shoots in a later stage of development, **(D)** rooted cutting. (Photos: A and C taken by Tuija Aronen, B by Karl-Anders Högberg, and D by Jörgen Hajek)

Rooting is performed on a heated bed that enables the temperature to reach +25°C in the substrate. The relative humidity is kept between 60-75%, while the photoperiod is set to 18h with a light intensity of 12000 lux. The cuttings are hormone treated by dipping the cutting base in 8000-12000 ppm solution for 5-10 seconds. A porous substrate is used to allow for irrigation to keep the relative air humidity in the desired interval.

The basic idea in the new project is to harvest cuttings twice on the same donor plant with the aim to produce ten ramets of each donor plant appropriate for planting in field tests.

Organisation of the work

Five alternative models are tried:

A. Two consecutive winter propagations, year 3 and year 4 after sowing, respectively.
B. A winter propagation year 3 followed by a late summer propagation the same year.
C. A spring propagation year 2 after shoot production in a heated greenhouse during winter, followed by a late summer propagation the same year.
D. Two consecutive late summer propagations year 2 and year 3, respectively.
E. One winter propagation year 4 after sowing.

Rooting environment factors (F) are studied in separate experiments including temperature, water regime and GA-inhibitor treatment.

The models are distributed on participants according to Table 1 below. Sweden is represented by Skogforsks research stations Sävar and Ekebo, Finland by the Metla research stations Haapastensyrjä and Punkaharju, Latvia by Silava institute in Salaspils.

Table 1. Distribution of the project work on the five participating units. Model labels are explained in the text above.

Model	Sävar	Haapastensyrjä	Punkaharju	Ekebo	Salaspils
A	x	x	x	x	x
B	x	x			
C	x		x		
D	x			x	
E	x				x
F	x	x	x		

The plant material consists of five families in common in all experiments and at all locations. Furthermore, 15 local families are included at each location. For the rooting environment studies a separate set of ten families at each location is included.

Beside the search for the best production-rooting compromise, important issues in the project are the genetic influence on propagation ability and possible propagation effects on growth habit.

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Working Papers of the Finnish Forest Research Institute 114: 48–52

Rooting Nordmann fir cuttings for Christmas trees?

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Recent succes with rooted cuttings in fir (*Abies* spp.) propagation stimulated this experiment with *Abies nordmanniana*, an important Christmas tree species in Denmark. Cuttings were taken in late summer from young and older trees, untreated or stumped, and from specified positions within the crown. Auxin was administered to some cuttings at varying concentrations. Rooting was monitored over a period of 6 months and attained 60-70% in the best shoot types. Auxin had no influence on rooting but concentration correlated positively with tissue decay in basal end of cuttings. Rooting of cuttings from the leader in untreated trees decreased markedly with age while cuttings from the branches decreased less dramatically. No basipetal trend of rooting capability of branches could be ascertained. Orthotropic shoots that regenerated on stumped trees rooted with varying success, the ones originating from the main stem, and preferably at a low position, performing best. Orthotropic growth was maintained in 35-45% of these shoots 8 months after rooting, while plagiotropic growth was prevalent in cuttings of other shoot types.

Keywords: *Abies*; aging; conifer; cloning; plagiotropy

Introduction

Nordmann fir *Abies nordmanniana* Spach. is a widely used Christmas tree species, mainly grown in short rotation, and marketed throughout Europe and to some extent also in North America. Traditionally, vegetative propagation by cuttings has not been considered a practical option for true firs (*Abies* spp.), and especially not for trees beyond a few years of age. Irregular rooting and strong persistency of the plagiotropic growth pattern have been major obstacles (Brandt 1979, Hocevar 1983). The plant material routinely used in production is thus seedlings, mainly based on direct seed imports from the natural range within the Georgian Republic in Caucasus and neighbouring countries along the western part of the Black Sea.

Inspired by recent rooting success in true firs (*Abies* spp.) (Rosier et al. 2004, 2005) and our newly gained knowledge of phytohormone levels in *A. nordmanniana* (Rasmussen et al. 2009), we decided to set up experiments for rooting Nordmann fir cuttings, primarily for the purpose of obtaining clones for various experimental work. The aims was to test rooting ability of cuttings from variously stumped and untreated mother plants, cuttings of different age and from different position within the tree, and finally to evaluate rooting performance and plagiotropy in relation to hormone profiles determined for selected phenotypes/response-types.

Material and methods

Two groups of plant material were used: Three years old (one branch whorl) and five years old seedlings (3 branch whorls growing to 4). The former were used for a study of auxin pretreatment (testing 0, 1.25, 2.5, 5 and 10mM NAA), and the latter for studying position effects and reactions of shoots regenerating after stumping. Three stumping treatments were applied: the stem reduced to the second-lowest branch whorl, to the lowest branch whorl, and untreated. Stumping took place in late April at which time all terminal buds on remaining branch whorls were also removed.

New orthotropic shoots emerged on stumped trees during spring and summer 2007, mostly on the stem and on the uppermost branch whorl. In August (23th) these shoots as well as plagiotropic branch shoots were excised as cuttings. Origin and position on the trees were carefully noted. A total of 539 cuttings were set in a moist Pindstrup II peat/perlite (2:1 by vol.) mixture. The culture was covered by a cage with white acrylic and kept indoors in a greenhouse at 20°C minimum temperature with ventilation at 25°C (Fig. 1). No misting was applied and very little additional watering was required during the rooting phase. Cuttings showing fungal infection were sprayed individually with 0.1% Octave fungicide. Supplemental light of app. 40 W/m^2 was given, maintaining a 20 hour day length until February 2008. Rooting was monitored on 30th October, 5th December, 6th February and 14th May. In early February all rooted cuttings were transferred to 10 cm containers with Pindstrup II and gradually subjected to a lower temperature at 5°C (ventilation at 8°C) and natural day length, where they were kept from 14th February to 9th April. The temperature was subsequently raised to 15°C (ventilation at 25°C) for bud breaking and seasonal growth in the green house.

Fig. 1. Experimental setup.

Plagiotropy of the rooted cuttings was assessed in August, 12 months after setting, when the new shoots had matured. The plantlets were grouped according to increasing plagiotropy, category A representing the fully orthotrophic and G the most pronouncedly plagiotropic (Fig. 2).

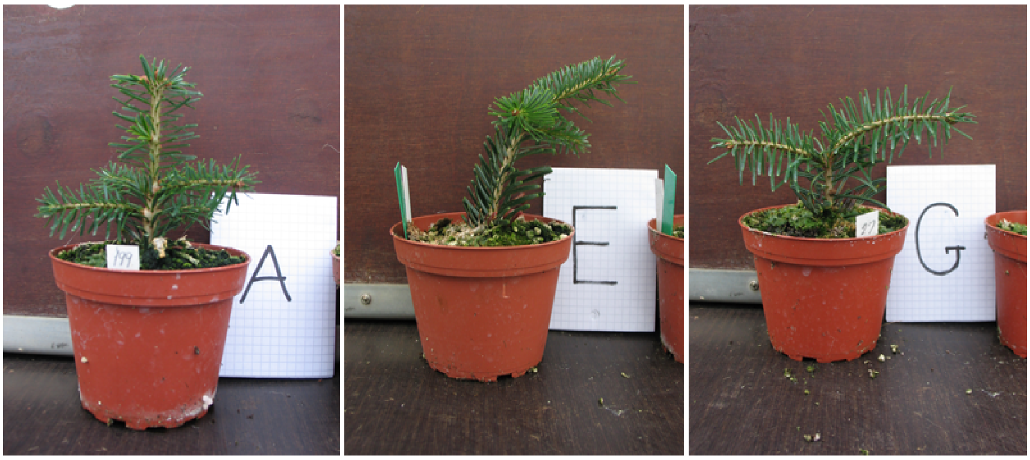

Fig. 2. Samples from the orthotropy-plagiotropy gradient used for evaluating rooted cuttings. Left: fully orthotropic, centre: intermediate, right: fully plagiotropic.

Results and discussion

On average, 68% of the cuttings from 3-y old trees rooted successfully, and 49% of those from 5 y old trees. Rooting took place slowly; an assessment 9 weeks after setting showed callusing at the cut surface in 60% of the cuttings but only root development in 20%. Even at the February assessment, about 6 months after setting, additional rooted cuttings were noted. In contrast to previous results in *A. fraseri* (Rosier et al. 2005), auxin treatment had no significant positive effect on rooting (Fig. 3). Rather, basal tissue decay and subsequent death seemed to correlate with increasing concentrations of NAA.

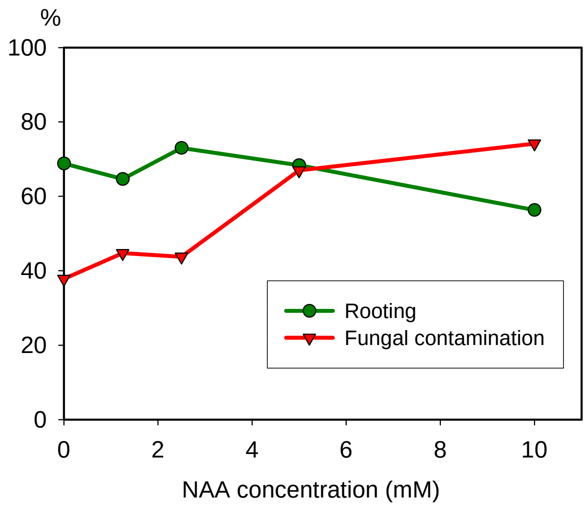

Fig. 3. Rooting percentage (green, circles) and basal cutting decay (red, triangles) as a function of auxin (NAA) concentration. Cuttings from ortets at the end of their third growth season, leader shoots only.

Leader shoots (from unstumped 3-y old trees) had 62% rooting and side branches from the uppermost whorl 74% (Fig. 4). In contrast, leader shoots from the 5-y old untreated plants had only a 10% rooting while side branches rooted 55-72%. Rooting capacity in leader shoot thus appeared to decrease dramatically with tree age, while rooting in whorl branches were less influenced. The lowermost branch whorl from the older trees showed the poorer rooting but there was no obvious basipetal or other positional trend, as seen in other studies (e.g. Hocevar 1983).

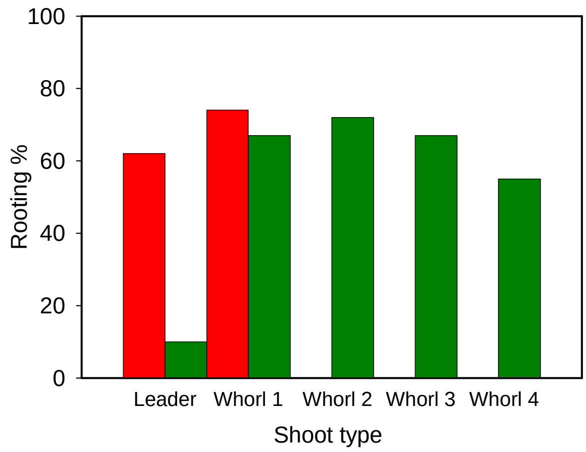

Fig. 4. Decrease in rooting with age, ortets 3 (red) and 6 (green) growth seasons old. Cuttings from whorls were the terminal shoots, the whorls being numbered consecutively from above.

Following stumping of the mother trees in April, which deprived them of their natural leader shoot, several branch reactions were seen. New shoots developed from needle axils on the upper side of the remaining whorl branches and on the stem above ("high") or below the whorl branch ("low"). Many of these shoots were orthotropic with respect to needle and bud orientation. In spite of the tip pruning of remaining whorl branches, some subdominant branch tips turned hyponastic ("raised branch"). Side shoots from whorl branches in a low position were used as reference shoots ("low side branch") in the rooting experiment of these shoot types. Rooting differed considerably among these types of cuttings, the orthotropic low rooting almost as well as the low side branches (60-70%, fig. 5). In contrast to the latter, raised branches appeared very difficult to root and tending to revert to plagiotropy. Plantlets originating from low side branch cuttings were 96% plagiotropic, while the orthotropic low cuttings gave about 42% orthotropic plantlets. Compared with orthotropic low shoots, orthotropic high shoots performed less well with respect to rooting and about as well with respect to orthotropy (Fig. 5).

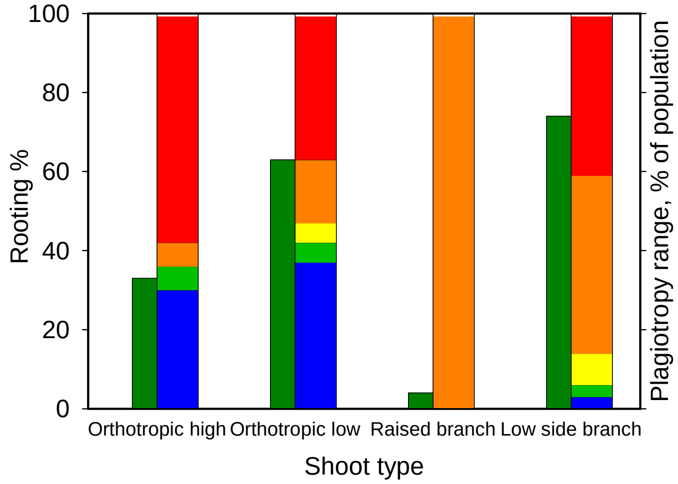

Fig. 5. Rooting percentage (slender green bars) and plagiotropy of resulting plantlets (multicoloured bars) in cuttings made from various shoot types developing after stumping of mother trees. Blue represents the fully orthotropic, red the fully plagiotropic, and green-yellow-orange intermediate types. Shoot types: cf. explanation in text.

Preliminary conclusions

- Cuttings taken in August were capable of rooting within 6 months at 60-70% in the best shoot types
- Auxin pretreatment had no positive effects
- Orthotropic shoots regenerating from stumped mother trees had a better chance of growing orthotropically as rooted cuttings
- Shoots regenerating from lower positions on the stem performed best

Future studies

When the cuttings were set, samples of the basal stem were taken and freeze stored (-80°C). Analysis of phytohormone profiles from selected response types is planned. A new batch of orthotropic cutting material was prepared by stumping of young and older (15-y) trees in February and a second set of cuttings was set, this time in mid July. Data from Rosier et al. (2004) indicated that softer summer cuttings performed better than semi-hard wood cuttings from autumn and hardwood cuttings sampled in early spring. This timing seems to agree with seasonal patterns of auxin and cytokinins, corresponding to an endogenous maximum in stem auxin and a minimum in cytokinins (Rasmussen et al. 2009).

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Working Papers of the Finnish Forest Research Institute 114: 53–54

Using buds from mature trees of *Pinus sylvestris* for budding young seedlings

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A budding method study for multiplying selected mature Scots pine trees
Young Scots pine (*Pinus sylvestris*) seedlings were budded by using buds from mature trees of the same species or from Arolla pine (*Pinus cembra*). The first pilot experiments were carried out in 1990 at the Michigan State University (USA) and later similar plant material was produced for environmental studies at the University of Oulu (Finland) and for forestry related studies for Metla (Finnish Forest Research Institute) and the Department of Applied biology of the University of Helsinki (Finland).

Material

Young seedlings (4 - 6 months) were used as rootstock. They were treated and grown equally to seedlings for forestry purposes. Different seed sources were used according to the availability of seedlings from forest tree nurseries or availability of seedlings from other Scots pine studies in Haapastensyrjä breeding station. Buds were collected from different Scots pine trees of more than 60 years of age in the spring or early summer before they started to sprout. All buds of Arolla pine were collected from one, more than 20 years old tree. All of the stock trees were healthy and their form was good, however, living in open places they had also branches growing close to the ground. All buds were collected from the lowest branches.

Methods

Two different methods were used: top budding (fig. 1) and side budding (fig. 2) (similar to top grafting and side grafting). Rootstock seedlings were approximately 10 cm high. In top budding method needles were removed from top of the seedlings and in side budding tops were saved but needles were removed below it to allow side cut and binding. Buds were collected with five centimeters of branch. The collected buds were used either directly or stored in cold room in plastic bags. Buds were prepared by using scalpels. All the attached wood was removed and the buds were cut to V-shape to fit in split cut of the rootstock seedlings.

The buds were bound and covered with Para-film. The budded seedlings were either grown outside protected from direct sunlight or in a growth chamber.

Fig. 1. A bud from a mature Scots pine tree has been budded into a young seedling of Scots pine using top budding method.(Photo: Tapani Haapala)

Fig. 2. Side budding method is used to combine Scots pine seedling with a bud from a mature Scots pine. It is time to remove original top of the seedling to allow the new shoot to develop into a new top of the plant. (Photo: Tapani Haapala)

Discussion

Altogether more than two hundred budded pine seedlings have been produced, most of which are Scots pine and about 30 Arolla pine. Budding is a very efficient and fast method which requires very little space. When using potted seedlings, more than 500 seedlings can be grown in a space of 1 m^2. In some experiments almost all of the buds started to grow. In one experiment, when bud material was not stored properly, none of the buds grew well.

No significant differences were found between the methods. However, top budding is a much easier and a slightly faster method than side budding. Most of the seedlings were used for environmental studies. However, some of the oldest trees are growing on research fields of the University of Oulu and are now over ten years old. The youngest ones are less than one year old and they are to be used for further studies of the method. The Scots pine seedlings when budded with Arolla pine buds grew as well as when budded with Scots pine buds.

Working Papers of the Finnish Forest Research Institute 114: 55–58

Field performance of rooted Scots pine cuttings of fascicular shoot origin

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Clonal testing could facilitate the breeding of Scots pine, but so far it has not been possible to apply it in practice due to difficulties in the vegetative propagation of this species. Several propagation techniques have been developed for Scots pine, but no reports on the field growth of vegetatively propagated material have been published. The aim of this study was to examine the field performance of 10- to 11-year-old Scots pine cuttings of fascicular shoot origin, rooted either with *Agrobacterium* or IBA hormone treatment. The rooted Scots pine cuttings were able to grow well in the field but the field performance varied according to the timing of rooting, quality of the induced root system, and genetic background of the cuttings. In order to achieve the true growth potential and proper growth habit of the cuttings, the donor plants should be as young as possible and IBA treatment is recommended for root induction.

Keywords: adventitious roots, *Agrobacterium rhizogenes,* fascicular shoots, field performance, indolebutyric acid (IBA), *Pinus sylvestris*

Introduction

Scots pine (*Pinus sylvestris* L.) is one of the key species of forest ecosystems over large areas of the boreal forest zone in Europe and West Siberia, and is of considerable economic importance. In the Nordic countries, Scots pine has been included in forest tree breeding activities since the beginning of the 1940's, but it has only recently reached the second breeding cycle. The main reason for the slow progress in genetic improvement is the drawn-out testing based on progeny trials following the rearing and crossing of selected candidates. If vegetative propagation of the candidates was possible, could clonal testing considerably facilitate breeding by shortening the time required for testing per generation.

No reports on the field performance of vegetatively propagated Scots pine material have been published. The aim of this study was to examine the field performance of 10- to 11-year-old Scots pine cuttings of fascicular shoot origin, rooted either with *Agrobacterium* or a hormone treatment (Aronen et al. 1996). In addition to focussing on the overall growth and morphology of the cuttings, the effects of the root induction treatments and genetic background of the cuttings on their field performance were also studied.

Material and methods

The material consisted of rooted cuttings of fascicular shoot origin of Scots pine (*Pinus sylvestris* L.), representing three mixtures of seed orchard seed and three specific crossings. The pollen parent in the controlled crossings was a special narrow-crowned form of Scots pine, E1101 (Fig. 1). The rooting of the cuttings was induced with IBA with or without different A*grobacterium*-treatments. The rooting treatments were repeated four times, twice a year, after an artificial growing season during winter in April and in June in the years 1993 and 1994 in the greenhouse. The cuttings were located outdoors, with spacing of 0.5 m, on sandy soil in 1998. The field performance of the cuttings was examined in 2004.

Fig. 1. The tree E1101 showing the tortuous stem. (Photo: Eija Matikainen)

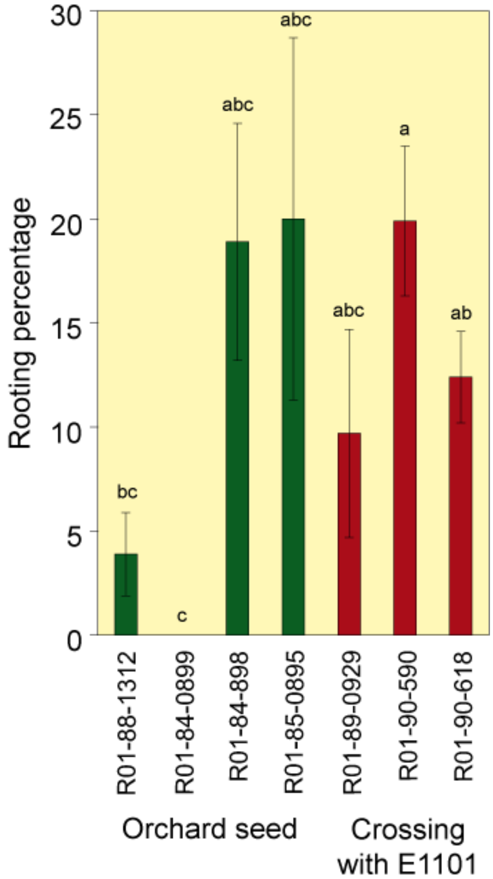

Fig. 2. Rooting percentages of different Scots pine cutting lots showing the means of all rooting treatments and times. Different letters indicate significant differences among the lots.

Results and discussion

Rooting percentage was better in June lots as well as using IBA with *A. rhizogenes* in both years. Also the genotypic background of the material affected the rooting success of lots with the average rooting percentage varying from 0 to 20 (Fig. 2).

Fig. 3. Growth of rooted Scots pine cuttings in a nursery trial. The cuttings are presented at the same scale, the label with the cuttings number indicating height of 1 m. (Photos: Leena Ryynänen)

Field performance of the rooted cuttings was variable (Fig. 3). The average height of the cuttings was 206 cm, varying from 331 to 60 cm. The bacterial treatments and IBA had no remarkable effect on height growth of the cuttings. The mean stem diameter at the base of the stem was 30 mm and dbh 17 mm. *A. rhizogenes* -mediated rooting resulted in inferior cuttings compared with the IBA treatment. In the cuttings given the bacterial treatment, the stem base diameter was 26 mm and the dry weight of the roots 111 g, while in the IBA-rooted cuttings the diameter was 36 mm and the dry weight of the roots 247 g. An average tilting of the stems was 27° varying from 0 - 80°. The April lot cuttings had the better stem angle (19°) accompanied with better increase in height in 2004 (36 cm) compared with the annual growth of June lot cuttings (31 cm) with more plagiotrophic tilting of 30°. Rooting or its timing had no influence on stem straightness of the cuttings.

The mean height of the present cuttings (205 cm) is within range of the average stem height of 10-year-old Scots pine standard seedling lots from the corresponding seedling lots, but seems low for selected plus tree material. Twirled roots caused by long-term pot culture, as well as relatively narrow spacing in the field may have inhibited the growth of cutting to some extend.

The genetic background of the cuttings i.e. the orchard seed lots or specific crossing with E1101 in which they originated, affected their growth. The annual height growth of the progenies of the former compared to the crossing progenies of E1101 was better in all the studied years (2002-2004) (Fig. 4A). Also the stem straightness was dependent on the genetic background. In the E1101 progenies there were more trees with badly tortuous stem than in the cuttings of the orchard seed lot (Fig. 4B).

The genetic inheritance of E1101, with unfortunate tortuosity of the stem clearly affects the fenotype of the cuttings propagated from the progenies of E1101. The feature is supposed to be caused by a single dominant allele (K), the tree itself being heterozygous (Kk) for it. In its crossings with wild-type (kk) the segregation in progenies is close to 1:1. This is in accordance with result in the present material, 43% of the cuttings propagated from E1101 progenies were completely tortuous.

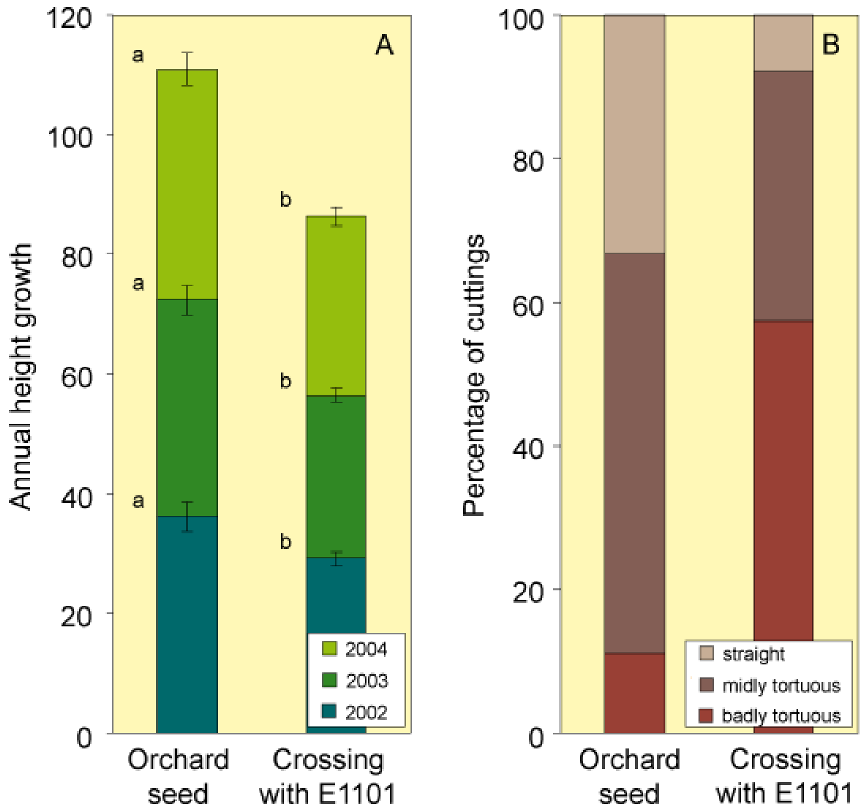

Fig. 4. Cuttings with different genetic background. **A**) Annual height growth in 2002-04. Different letters indicate significant differences between the groups. **B**) Frequency of the cuttings having a straight, mildly tortuous, or badly tortuous stem.

In conclusion, the rooted Scots pine cuttings were able to grow well in the field but the field performance of the cuttings varied according to the timing of rooting, quality of the induced root system, and genetic background of the cuttings. In order to achieve the true growth potential and proper growth habit of the cuttings, the donor plants should be as young as possible and IBA treatment is recommended for root induction.

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Working Papers of the Finnish Forest Research Institute 114: 59–63

Effect of stock plant pruning, hormone treatments and stock plant origin on the height of cloned Scots pine in a field trial

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The effects of stock -plant pruning, treatment with plant hormone sytokinin 6-bentsyladenin (BA) using three concentrations (8 x 0.25 mM, 8 x 0.5 mM and 4 x 1 mM) and auxin indole-3-butyric acid (IBA 0,5 mM) treatment to the height growth of of the Scots pine (*Pinus sylvestris* L.) was determined in a 10-year field trial with cuttings (n=836) from four seed orchard origins (half-sib families) and four controlled crosses (ful-sib families). Both pruning and sytokinin treatment had a significant effect on the height of the cuttings, but no effect of IBA treatment could be detected. The evening out of the differences in planting height at the beginning of the field trial and the differences between the origins at the end indicate that the genetic origin of the cuttings was a significant factor affecting the height growth of the Scots pine rooted cuttings in this field trial.

Keywords: Scots pine, *Pinus sylvestris*, cuttings, field trial, height growth, pruning, hormone treatment, genetic origin

Introduction

There have been conflicting reports on the height growth of cloned pines compared to seedlings in field trials (Pawsey 1971, Sweet & Welles 1974, Struve et al. 1984, Struve & McKeand 1990, Stelzer et al. 1998, Niskanen et al. 2008). The observed variations have been explained by the planting size of the cuttings, by the age of the stock plants, by the size of the root system in cuttings by planting and by the genetic origin of the cuttings (Frankling 1969, Foster et al. 1985, 1987, 2000, Struve and McKeand 1990, Niskanen et al. 2008). The growth of the rooted cuttings of Scots pine in the field has been reported to be adequate (Aronen & Ryynänen 2005, Niskanen et al. 2008). In this experiment our objective was to determine how stock plant pruning, treatments with sytokinin (for shoot elongation) and auxin (for rooting of the cuttings) and stock plant origin affected the height growth of Scots pine (*Pinus sylvestris* L.) rooted cuttings in a 10-year field trial.

Material and methods

Young (2 and 5 years old) stock plant (N=39) seedlings from four seed orchard origins (half-sib families) and four controlled crosses (ful-sib families) - pruned or not pruned - were sprayed in the spring of 1989 with different BA concentrations: 8 x 0.25 mM, 8 x 0.5 mM and 4 x 1 mM for four weeks. In 1990 all shoots that were elongated 1.5 cm or more were cut and soaked in 0.5 mM IBA solution for 20 hours in the dark for rooting.

A field trial of 836 rooted Scots pine cuttings was established in 1992 in Finnish Forest Research Institute, Haapastensyrjä Tree Breeding Station, Loppi, Finland. The experimental design was completely randomised test of single ramet plots (1.8 m x 1.8 m). The number of cuttings/stock plant varied between 10-56. The height of the cuttings was measured in summer 1993, 1994, 2001 and early in the spring 2003. The effects of pruning, hormone treatments and stock plant origin on height were analysed by one-way analysis of variance. The initial planting height was used as a covariate in analysis of the effects of BA treatment and stock plant origin.

Results

Pruning

Cuttings from the pruned stock plants were shorter than the cuttings from not-pruned stock plants during the whole experiment in 1993-2003 (Fig. 1) and the height difference was significant (Table 1).

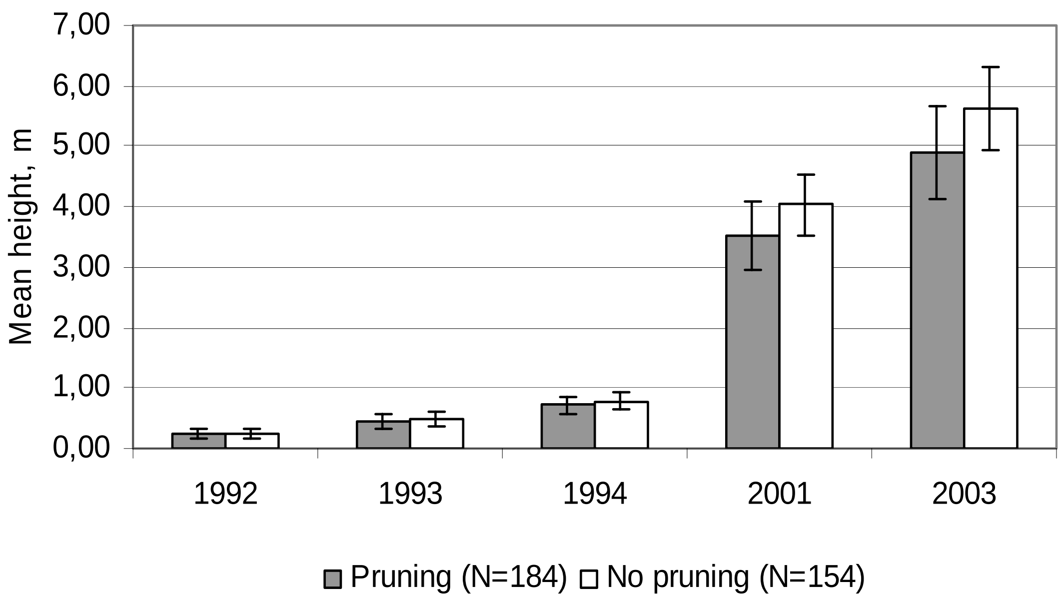

Fig. 1. The mean heights of rooted cuttings of Iitti seed orchard stock plants (7 pruned, 6 non-pruned), all treated with 8 x 0.5 mM BA. Bars represent standard deviation.

BA treatment

Cuttings from stock plants treated with 8 x 0.5 mM BA were the shortest of the cuttings treated with three different hormone concentrations (Fig. 2). The differences were significant between treatments throughout the whole experiment (Table 1). When the Tukey's test was used in pairwise comparisons of the treatments it was the 8 x 0.5 mM BA treatment which differed from the other two.

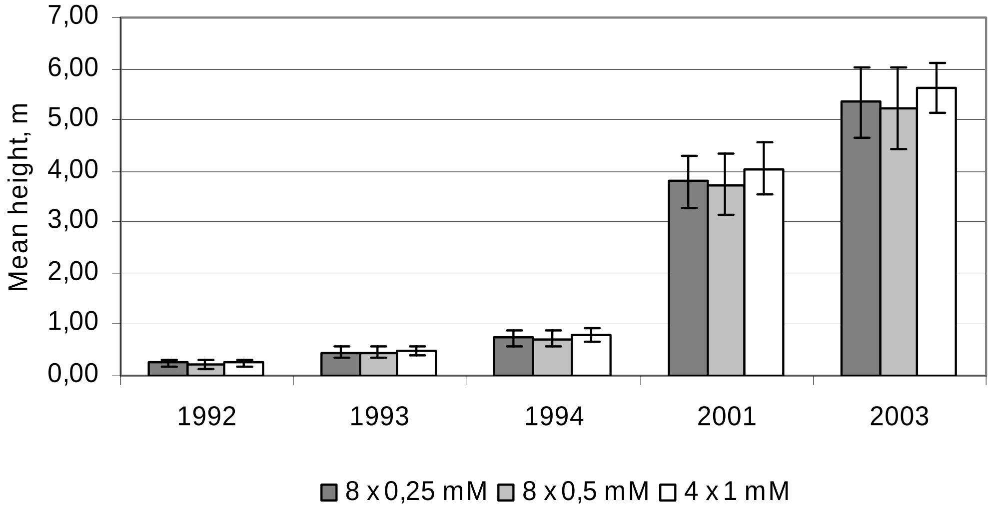

Fig. 2. The mean heights of rooted cuttings treated with different BA concentrations: 8 x 0.25 mM (N=48), 8 x 0.5 mM (N=684) and 4 x 1 mM (N=104). Bars represent standard deviation.

IBA treatment

The cuttings rooted with IBA treatment seemed to be slightly taller in 2001 and 2003 than the cuttings which were rooted without auxin treatment (Fig. 3). However, the possible difference could not be detected statistically (Table 1).

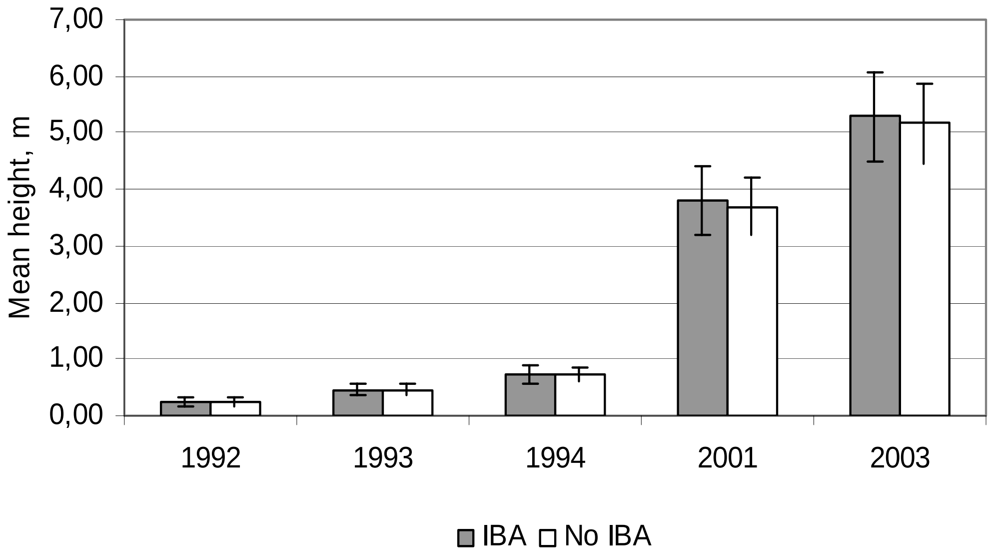

Fig. 3. The mean heights of IBA treated (N=744) and without IBA (N=92) rooted cuttings. Bars represent standard deviation.

Stock plant origin

Significant differences in height were observed between different stock plant origins at the beginning (1992 and 1993) and at the end (2001 and 2003) of the experiment (Table 1) when the cuttings with 8 x 0.5 mM BA treatment were used from each origin.

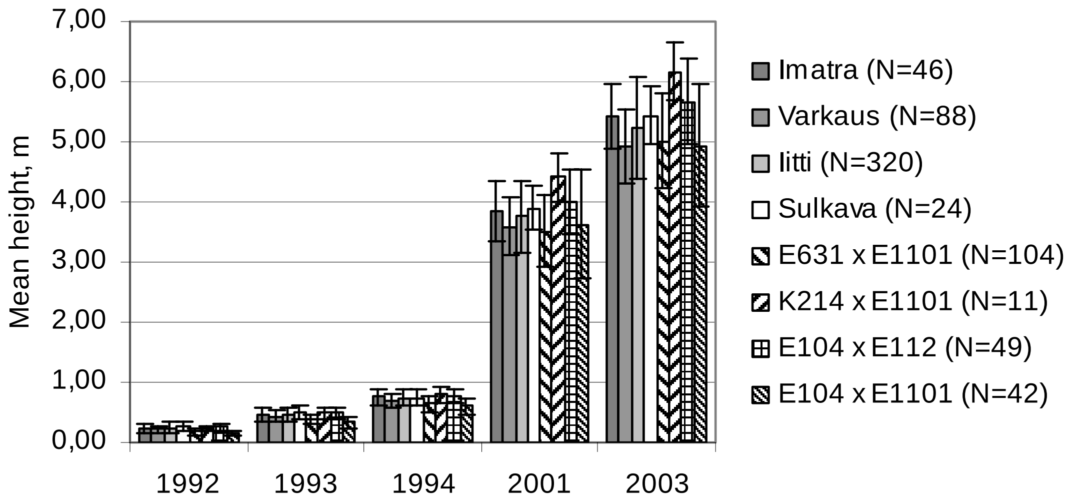

Fig. 4. The mean heights of rooted cuttings from 8 different stock plant seed origins. Bars represent standard deviation.

Table 1. One-way analysis of variance on the effects of stock plant pruning, BA treatment, IBA rooting treatment and stock plant origin on height in a field trial in 1992-2003. The initial planting height was used as a covariate in the analysis of the effects of BA treatment and origin in 1993-2003.

Source of Variation	df	1992		1993		1994		2001		2003	
		F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Pruning	1	0.444	0.505	17.392	<0.001	21.247	<0.001	75.228	<0.001	84.921	<0.001
BA treatment	2	4.075	0.017	7.492	0.001	9.173	<0.001	12.212	<0.001	11.923	<0.001
IBA treatment	1	3.564	0.059	0.485	0.487	0.607	0.436	0.085	0.771	0.509	0.476
Origin	7	25.463	<0.001	2.704	0.009	2.319	0.240	1.441	<0.001	10.876	<0.001

Conclusions

The height growth of the rooted Scots pine cuttings in this experiment was affected by stock plant pruning and by sytokinin treatment but the effect of IBA treatment for root development could not be detected. The height of the cuttings of different origins varied by planting indicating that the sytokinin treatments may have different effects on different genotypes. The difference by planting started to even out after one growing season, but was statistically significant at the end of the experiment in 2001 and 2003, indicating that the genetic origin of the stock plant was the main factor determining the height growth of Scots pine clones in the field.

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Working Papers of the Finnish Forest Research Institute 114: 64–67

Production of Norway spruce somatic embryos − a practical point of view

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Somatic embryogenesis is a sequence of operations with technical, biological and logistic aspects. The technical challenges are encountered mainly in the automation of the processes, most important the steps where somatic embryos and plantlets/plants are handled one-by-one, i.e. partial desiccation, germination and acclimation. Biologically, the maturation-desiccation-germination sequence is a very critical issue, and lack of root formation seems to be a major problem. A protocol that produces somatic embryos with high quality and a high germination rate from many cell lines is desirable but not so easy to achieve. Sorting procedures will probably be necessary, of embryos before germination and of plantlets after germination. Furthermore, cryopreservation is experienced as a bottleneck and methods for preserving more tissue samples per time unit is wanted.

Keywords: automation, plant production, somatic embryogenesis

Background

Somatic embryogenesis is a vegetative propagation method with attractive features and during the two decades that has passed since the process was described the first time for Norway spruce a lot of research and development have been made. The two most important advantages of somatic embryogenesis is the cryopreservation option and the rapid multiplication of genotypes.

In principal, there are two propagation alternatives at hand when applying vegetative propagation: 1. propagation of many genotypes and few plants per genotype, typically for clonal testing, 2. propagation of few genotypes and many plants per genotype, typically for mass propagation of tested clones. Bulk propagation, i.e. propagation of untested clones, takes a position in between but is closer to alternative 2 than alternative 1. The two alternatives provides to some extent different requirements for the practical propagation work.

Procedure bottlenecks and their nature

The very first activity in a somatic embryogenesis propagation is excision of the zygotic embryo. If the protocol involves immature embryos the excision is preceded by dissection of cones. Regardless of the age of the zygotic embryos, it is hard to see any automation of this work. This position holds also for the next step, initiation, when the excised embryos are put on initiation medium. But the next step, proliferation of successfully initiated cell lines, can be performed in vessels with liquid media, s.c. bioreactors, that already are in use by e.g. CellFor. Proliferation is the growth phase of the propagation, and a rapid growing culture grows exponentially, which means that the workload increases dramatically in late stages of the propagation. Using bioreactors thus reduces the manpower needed for propagating big amounts of tissue.

To automate maturation is more difficult but not impossible, e.g. can tissue be maturated on membranes on liquid maturation media. However, when somatic embryos have matured the propagation moves into handling embryos one-by-one, in partial desiccation and germination. This is where automation is most beneficial as it reduces time-consuming manual labour, but it is also a big challenge. The key is how to find a technique that speeds up the handling without taking risks in damaging the small embryos. The idea of an artificial seed shell to protect the embryo and making it easier to handle has been proposed but so far no product has entered the market. Unprotected embryos can be handled by properly designed robots but also in this case no commercial product has been presented. Another aspect of the maturation-desiccation-germination steps is that there is a need of sorting. Either sorting of the mature somatic embryos to get a high probability of each embryo to produce a plant, or sorting after germination combined with transplanting of germinating plants. Root formation during germination appears to be an important issue in this context.

Finally, automatic handling would be beneficial for cryopreservation as well. However, a shortening of the time for preparation of samples that will go into storage would also be an important step forward.

Generally, propagation for clonal testing is more tolerant to manual operations and the resulting higher costs, as they will be diluted by a high number of plants per clone once the clone is selected. Mass propagation of selected clones will however be dependent upon an automatic procedure that can bring the costs down to reasonable levels. Similarly, propagation of a bulk material needs automatic handling.

Multiplication and deployment aspects

One restriction of somatic embryogenesis is that genotypes react differently with different protocols. To maximise the number of cell lines that will be produced in a propagation event, several protocols should be used. But for practical reasons normally only one standard protocol is followed. This results in very skewed distributions of clones per family and plants per clone (Fig. 1). The number of rooted cuttings varies considerably among clones and often a minority of the propagated clones contribute with a majority of the produced plants.

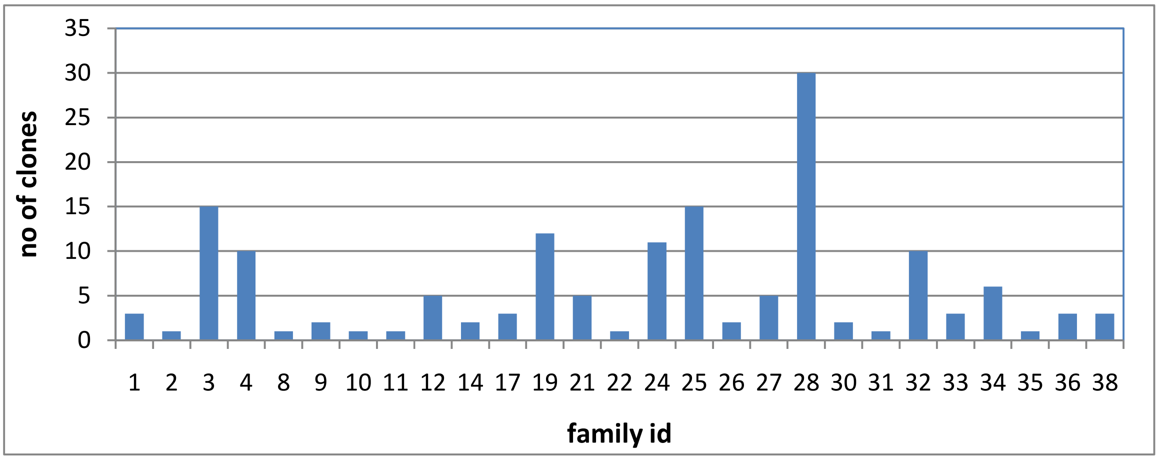

Fig. 1. Example of uneven distribution of clones among families.

Selection of superior clones after field testing is based on traits like growth, wood quality etc. in some kind of selection index. With somatic embryogenesis, a propagation trait will be added to the selection. Practically, this means that propagation ability will be a part of the index. Clones that are difficult to propagate in high numbers will not be selected even if the field performance is superior. Including one more trait will thus mean that more clones (zygotic embryos) needs to be included in the initiation to achieve the same genetic gain.

The other option of deployment, bulk propagation of superior families where a set of untested clones from selected and tested families is mass propagated, must also consider the restrictions mentioned above. Normally a larger number of cell lines is necessary to reach a high probability that the mean of propagated clones corresponds to the mean that can be calculated from the predicted family values. If the family response to somatic embryogenesis is unknown the insurance is, once again, to include more families.

Where will the breakthrough come?

Undoubtedly, the one-by-one handling of embryos and germinants is the most important issue in somatic embryogenesis development (Fig. 2).

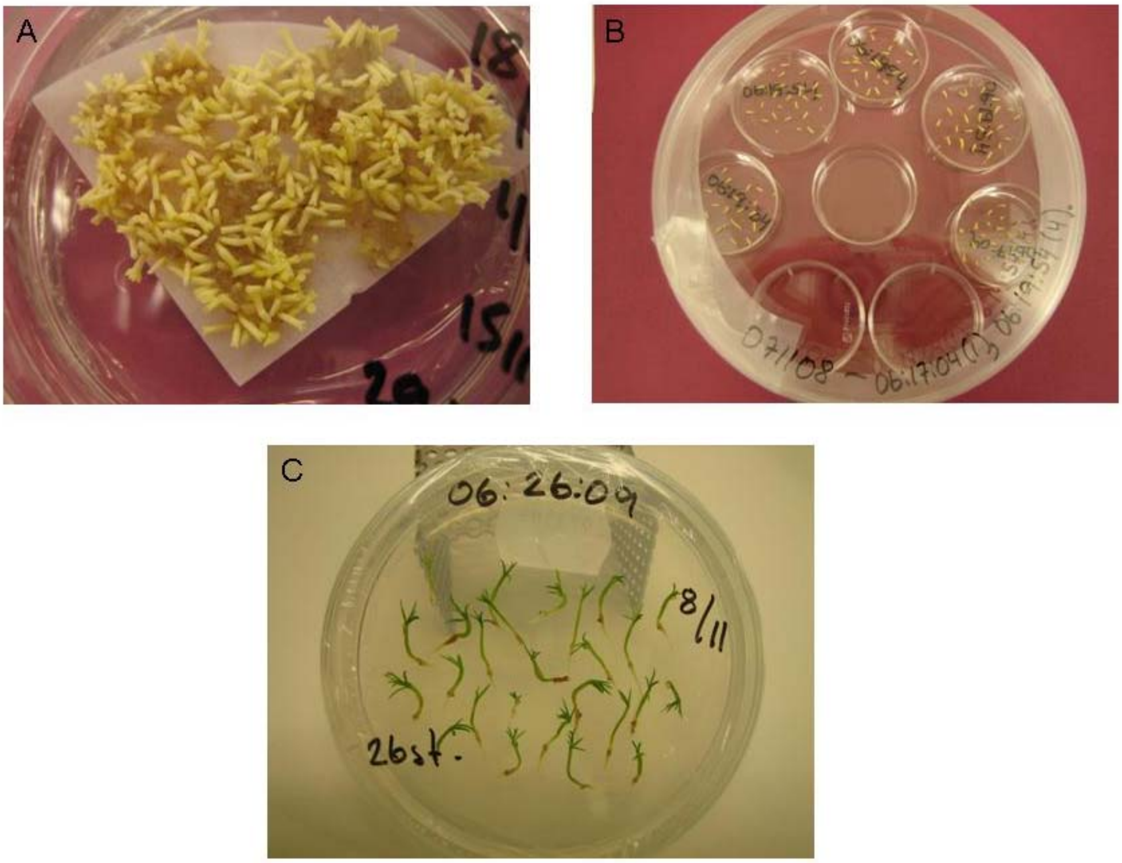

Fig. 2. Maturation **(A)**, partial desiccation **(B)** and germination **(C).** The stages in somatic embryogenesis where automation would be the breakthrough for large scale application of somatic embryogenesis. (Photos: Karl-Anders Högberg)

It is hard to see wide-spread large-scale applications without solution of this problem. Other important improvements may come as the biological understanding increases, like an improved standard protocol with higher probability of proper germination. More efficient cryopreservation procedures are also important to develop, but the crucial point is the automation of maturation-desiccation-germination stage and it is here we can expect the breakthrough in somatic embryogenesis to come.

Working Papers of the Finnish Forest Research Institute 114: 68–71

Somatic embryogenesis of Scots pine – advances in pine tissue culture at Metla

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Somatic embryogenesis (SE) is the vegetative propagation method providing the best multiplication factor for coniferous species. The technique has been applied in Scots pine (*Pinus sylvestris* L.) since the 1990's, but several steps of the method still need improvement. Research efforts at Metla's Punkaharju Research Unit have been focused, firstly, on enhancement of seed-embryo derived SE in Scots pine, and secondly, on cloning of mature Scots pine trees through SE. Recent advances and on-going work for developing somatic embryogenesis in Scots pine is described.

Keywords: zygotic embryo explant, mature tree explant, *Pinus sylvestris*, vegetative propagation

Introduction

World-wide, clonal or varietal forestry is advancing fast at the moment, because of evident gains achieved by multiplication of the very best individuals for silvicultural purposes. The biggest problem counteracting this development is the recalcitrance of mature conifers for vegetative propagation: there has been no way to clone well-known, selected adult trees having superior growth and wood qualities.

Scots pine (*Pinus sylvestris* L.) has been involved in forest tree breeding since its beginning, but enhanced clonal testing and silviculture options have been unattainable due to recalcitrance of the species in vegetative propagation. Rooted cuttings could provide an option for clonal testing in breeding programmes, but the most potential method for producing large amounts of clonal material is tissue culture through somatic embryogenesis (SE).

The SE techniques developed and used for coniferous species start generally from young explants, and in the case of Scots pine, from immature seed embryos. Research and development efforts for seed-embryo derived somatic embryogenesis in Scots pine has been going on at Finnish Forest Research Insititute (Metla), Punkaharju Research Unit, since 1990's.

Initiation and establishment of embryogenic cultures from vegetative shoot apices of mature trees of conifers has been attempted in a few species. Recently, Dr. Ravindra Malabadi and his co-workers have published reports on successful regeneration of SE plants starting from mature tree explants e.g. in *Pinus patula* (Malabadi & van Staden 2005), *P. roxburghii* (Malabadi & Nataraja 2006), *P. wallichiana* (Malabadi & Nataraja 2007), and *P. kesiya* (Malabadi et al. 2004). In collaboration with Dr. Malabadi, an attempt to apply this technique to Scots pine was initiated in 2006 at Metla's Punkaharju Research Unit.

Seed-embryo derived somatic embryogenesis

The method for somatic embryogenesis of Scots pine developed at Metla (Häggman et al. 1999) uses immature zygotic embryos taken from immature cones and surrounded by megagmentophyte as explants (Fig. 1A). In the recent years, research efforts have been focused to study the possibilities to improve the different steps of Scots pine somatic embryogenesis, i.e. initiation frequency, proliferation rate, production of mature somatic embryos, and conversion of embryos into plants. Special attention has been paid on embryo quality and root formation and their connections to preceding treatments. Also performance of the produced somatic embryo plants in greenhouse has been observed, and the plants will be further tested in field experiments starting in 2009.

The results achieved for enhancement of seed-embryo derived SE in Scots pine are described in detail by Aronen et al. (2009) and shortly summarised here. Genetic background has significant effect on initiation of somatic embryogenesis: Initiation frequencies of 20-30 % can be achieved, but there are also families with very low or no initiation. Proliferation of the established SE cultures is better with tissues suspended on filters than when they are grown as calli. Proliferation method also has an effect on the abundance of proembryos in the cultures, as well as on the number and quality of the mature somatic embryos later on. The most important factor for embryo production is, however, maturation technique used. Specific attention should be paid on quality of the somatic embryos produced, slim-type embryos having the best germination and greenhouse survival.

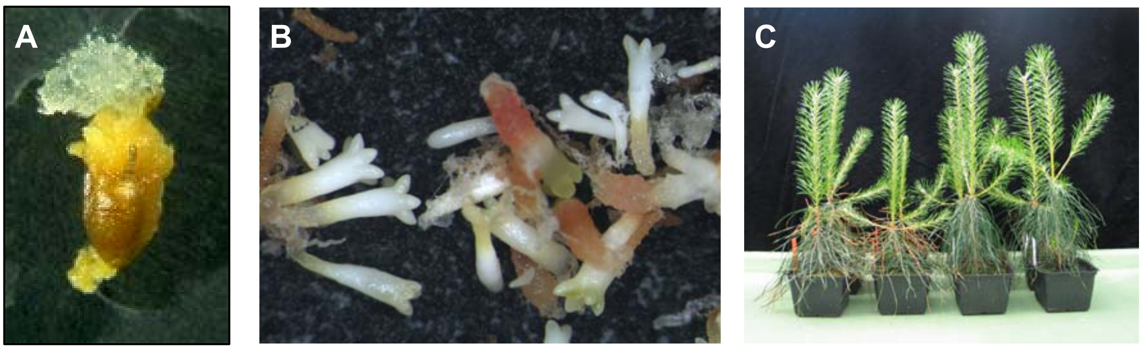

Fig. 1. Somatic embryogenesis in Scots pine: **A**) Induction of embryogenic tissue from immature seed embryo explant. **B**) Mature somatic embryos ready for germination. **C**) Somatic embryo plants on left and seedlings on right. (Photos: Jouko Lehto and Tuija Aronen)

To conclude, in order to get high-quality somatic embryos (Fig. 1B) that also perform well as emblings in greenhouse (Fig 1C), the SE cultures should be proliferated and matured suspended on filter paper, with high abscisic acid concentration applied during maturation. Mature somatic embryos should be picked up for germination only for a limited period, and their germination performed *in vitro* on tissue culture media. With optimised conditions and quality control of the embryos, over 90% of the somatic embryos germinate and develop into well-growing emblings.

On-going studies with shoot explants

In the last couple of years, research efforts have been focused on application of the Malabadi's published SE method (Malabadi & van Staden 2005) using explants from mature Scots pine trees. Tissue culture initiations using shoot apex explants from 10- and 15-year-old Scots pines were done through growing season to find right timing for SE induction. During a period in spring the explants seem to be responsive, and embryogenic cultures have been raised.

For initiation of somatic embryogenesis in shoot explants, distinguishing and separation of SE tissue from explant is of crucial importance. The explants need to be examined with stereomicroscope to find potential SE-tissue. Furthermore, acetocarmine staining should be used to evaluate embryogenic potential of induced tissue, and to avoid proliferation of non-embryogenic callus (Fig. 2A), often mixed with embryogenic one (Fig. 2B).

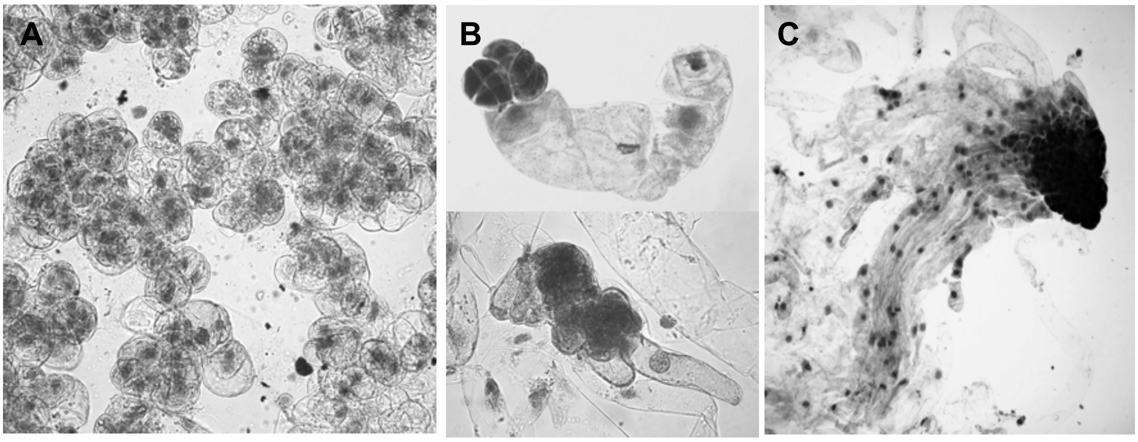

Fig. 2. Acetocarmine staining of tissues induced in shoot explants. **A**) Non-embryogenic cells with large cytoplasm. **B**) Early somatic embryos with strongly stained head cells and a few transparent suspensors. **C**) Well-formed embryo in proliferating SE-culture. (Photos: Ravindra Malabadi, Tuija Aronen and Leena Ryynänen.)

The majority of established SE lines proliferate well, and their recovery from cryopreservation has been 100% using the protocol developed at Metla (Häggman et al. 1998). According to microscopical observations, the proembryo formation in the most of the cultures is abundant, and the embryos are well-formed with complete head and prolonged suspensors (Fig. 2C).

Maturation and germination of somatic embryos in the cultures originating in mature trees has, however, been found to differ from the SE-lines of seed embryo origin. The first maturation experiments done according to the Malabadi's published protocols (Malabadi & van Staden 2005) resulted in very slow production of a few embryos, although the same method works for the Scots pine SE lines of seed-embryo origin (Aronen et al. 2009). Moreover, the quality of the embryos produced from the SE-lines of mature tree origin was not satisfactory (Fig. 3A), and they failed to germinate. Optimisation of maturation and germination steps is currently performed in collaboration with Dr. Marie-Anne Lelu from INRA, France. In the latest experiments, somatic embryos of good quality have been produced (Fig. 3B). In addition, marker analyses are performed to assess the genetic fidelity of the SE cultures originating in the mature trees.

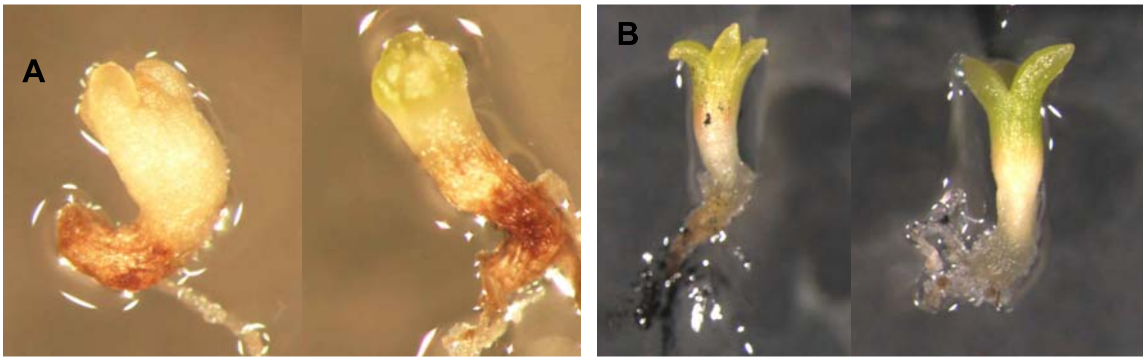

Fig. 3. Somatic embryos produced from SE lines originating in mature tree explants: **A**) Bad-quality embryos, and **B**) good-looking embryos. (Photos: Tuija Aronen)

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Integration of biotechnology, robot technology and visualisation technology for development of methods for automated mass production of elite trees

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Clonal propagation of elite trees by somatic embryogenesis can shorten periods needed for breeding of trees, and can ensure a stable production of high quality plants for the forestry sector. It will furthermore allow for relative fast market oriented breeding and the production of trees 'fit for purpose' and guarantee a consumer oriented and tailor made wood supply. However, commercial application of the technology has until now been hampered by two essential problems: 1) the production costs per plant must be reduced, 2) improved methods must be developed for transfer and acclimatisation of plants from sterile *in vitro* conditions to non sterile (*ex vitro/in vivo*) conditions in the nursery.

To solve these problems, a Danish based project has been established to combine clonal propagation by somatic embryogenesis (SE) with biotechnological breeding tools, and with robot – and visualisation technologies. The present project takes advantage of effective methods developed at the University of Copenhagen for SE in nordmanns fir and sitka spruce. These methods are used as a model system for development of biotechnological breeding tools in combination with automated plant production of plants for the forestry industry.

Introduction

Biotechnology has become an integrated part of plant breeding, and in recent years new methods have been developed for the breeding and propagation of important plants in the agricultural-, ornamental- and forestry sector.

One of the promising methods is somatic embryogenesis (SE), where plants are produced from single cells without sexual reproduction. SE has some particular advantages for the development of cost effective methods for clonal mass propagation of elite plants:

- It is a very effective and fast method for clonal propagation.
- The method is suitable for automatisation and robot technology.
- The method is, for several plant species, the preferred basis for development of additional biotechnological breeding technologies as e.g. genetic transformation.
- Elite clones can be stored for extended periods in liquid nitrogen at -196°C

However, commercial application of the technology has until now been hampered by two essential problems:

- The production costs per plant must be reduced. Labour costs are low in the early steps of the process whereas they increase dramatically during the later stages (Fig. 1).
- Improved methods must be developed for transfer and acclimatisation of plants from sterile *in vitro* conditions to non sterile (*ex vitro/in vivo*) conditions in the nursery.

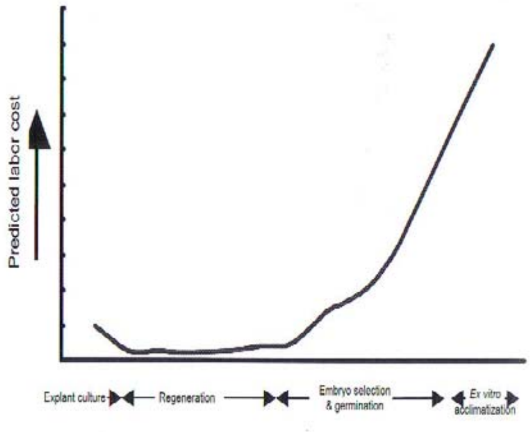

Fig. 1. Predicted labour cost by clonal propagation through somatic embryogenesis.

The labour costs are very low in the early steps of the production, whereas they increase dramatically during the later stages: 'embryo selection & germination' and 'ex-vitro acclimatization'). The aim of the present project is to reduce labour costs associated with the late stages of the production of cloned plants through development of robot- and visualisation technologies. (From Afreen & Zobayed, p. 96, 2005)

Model species

This study is based on effective methods for SE in nordmanns fir (*Abies nordmanniana*) and sitka spruce (*Picea sitchensis*) developed at the University of Copenhagen. The two species is of special interest for Danish forestry:

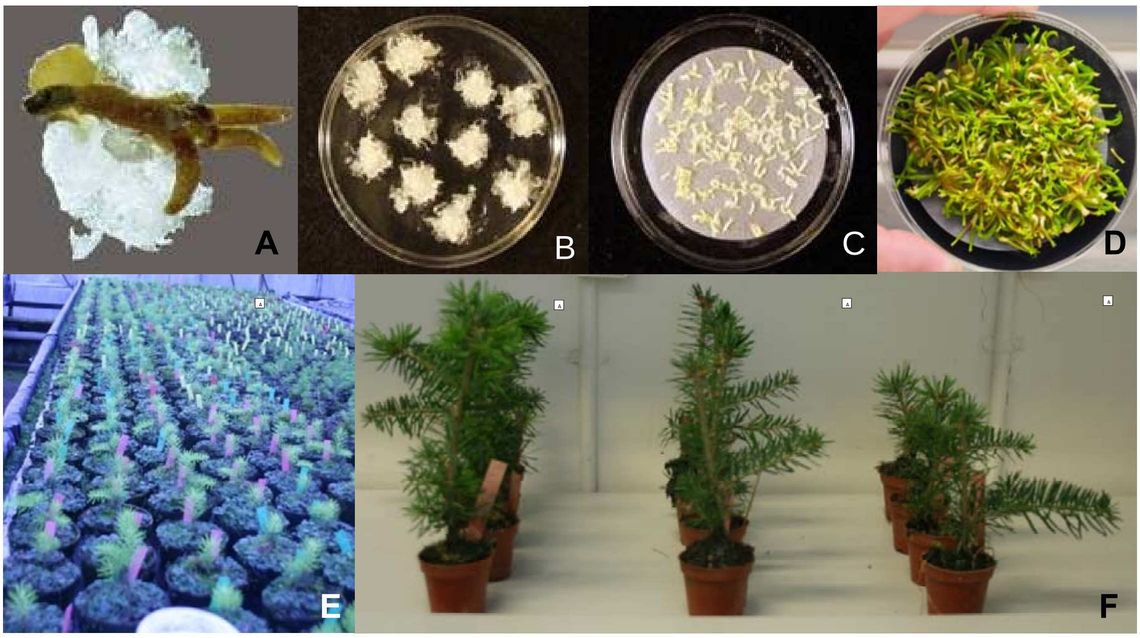

Fig. 2: Clonal propagation of conifers by somatic embryogenesis (Nordmanns fir): **A**: Embryogenic cultures are initiated from zygotic embryos, which are produced from controlled crossings of elite material. **B**: The embryogenic culture. The somatic embryos proliferate continuously and one Petri dish contains several thousand somatic embryos. The number of embryos doubles in approx. two weeks. At this stage it is possible to store the cultures over extended periods in liquid nitrogen (-196°C) and/or to ad new traits by gene technology. **C**: Mature somatic embryos are identical to mature embryos from seeds. **D**: Germinated embryos. **E**: Plants 12 month after transfer to soil **F**: Three different clones of nordmanns fir, phenotypically distinguishable after 2 years in soil.

Nordmanns fir for the production of Christmas trees

Forest trees are almost exclusively produced from seeds. For Nordmanns fir the seeds are collected in the natural forests in the Caucasus region in Georgia or from Danish seed orchards. The quality of the imported seeds are often unpredictable, and the production of high quality seeds are unstable from both Danish and foreign sources.

Nordmanns fir has a generation time of 25-30 years, and traditional breeding programmes is for this reason extremely time consuming. The extended generation period is a general problem in breeding programmes for forest trees, but it is particularly a problem in specialized industries that is dependent on fast breeding and development of new products. For this reason, the production of Christmas trees is hampered by plants with unpredictable genetic traits. The result is that only 15% of the produced trees are of best quality. Approx. 60% of the trees are of average or lower quality, and approx. 25% of the produced trees in a stand are discarded because they are not suited for sale (Table 1).

Table 1. Nordmanns fir. An example of economical gain from integration of cloning techniques in breeding programmes of forest trees. Distribution of trees in categories of quality and estimated sales price for 100 trees for plants produced from seedlings or by clonal propagation of elite material.

Sales price (D.kr.) for Christmas trees by use of clonal propagation

Category	Seedlings %	Cloned %	Price per tree D.kr.	Seedlings Sales price per 100 trees	Cloned Sales price per 100 trees
Superior	15	60	90	1350	5400
Standard	40	20	55	2200	1100
Class III	20	5	30	600	150
Discharged on form	20	10	0	0	0
Discharged on damage	5	5	0	0	0
Total	100	100		4150	6650

Average gain per tree by cloned material is approx. 25 D.kr. (approx. 3€)

Clonal propagation of elite material will ensure that the proportion of high quality trees increases and that the average price per produced tree is improved (Table 1). Therefore there is considerable interest in the development of methods for clonal mass propagation of elite trees. Traditional methods such as propagation by cuttings have been difficult for nordmanns fir due to poor rooting and plagiotropic growth. At present, SE is therefore the most promising method for enhancing gains from tree breeding programmes and for bulk propagation of elite trees.

Sitka spruce for production of biofuels

Sitka spruce plantations is characterized by a low input forestry as regards to management, harvest operations, application of herbicides, pesticides and fertilizers and the wood of Sitka spruce is of higher density compared to species such as willow and poplar. Furthermore, emission of NOX, HCL, and dioxin from wood combustion is low compared to straw, cereals and grasses (Obernberger et al. 2006). Incentives are therefore high for optimizing the production of wood for combustion and ethanol production.

At present the wood from Sitka spruce is mainly valuable for combustion since the amount and composition of lignin of conifers is more difficult to separate from the cellulose compared to other genera (e.g. Saddler et al. 2006). Nevertheless, ongoing research (e.g. Xue Jun et al. 2005, Saddler et al. 2006) may make it economically feasible to make ethanol from conifers in the future.

Vegetative propagation of elite trees identified for bio fuel production will improve the output significantly. Especially, when production is based on few clones as shown in a clonal field trial with 23-year-old Sitka spruce where the production of dry matter for one of the clones was twice the dry matter production of a standard Sitka spruce stand (Costa e Silva et al. 1994).

The results from the Danish breeding program (Roulund 1990, Costa e Silva et al. 1994, Hansen & Roulund 2001) can be combined with efficient biotechnological based methods for clonal mass propagation by SE (Fig.3) (Krogstrup et al. 1988, Find et al. 1993, Kristensen et al. 1994). Sitka spruce has been emphasized by OECD as a 'target species' for implementation of biotechnology in breeding programs for forest trees (OECD 2002).

Fig. 3. Sitka spruce field trial with trees produced by somatic embryogenesis. Established in 1991. (Photo Michel Kristensen, 2003)

Integration of biotechnology and robot technology in SE

Robots – visualisation – automatisation

The robot technology is integrated in two particularly labour consuming processes:

A) Isolation, selection and handling of mature somatic embryos (Fig. 5)

- Development of visualisation and handling technology for quality assessment, sorting and orientation of 3-5 mm long mature embryos.
- Development of technologies for transfer of embryos to germination medium.

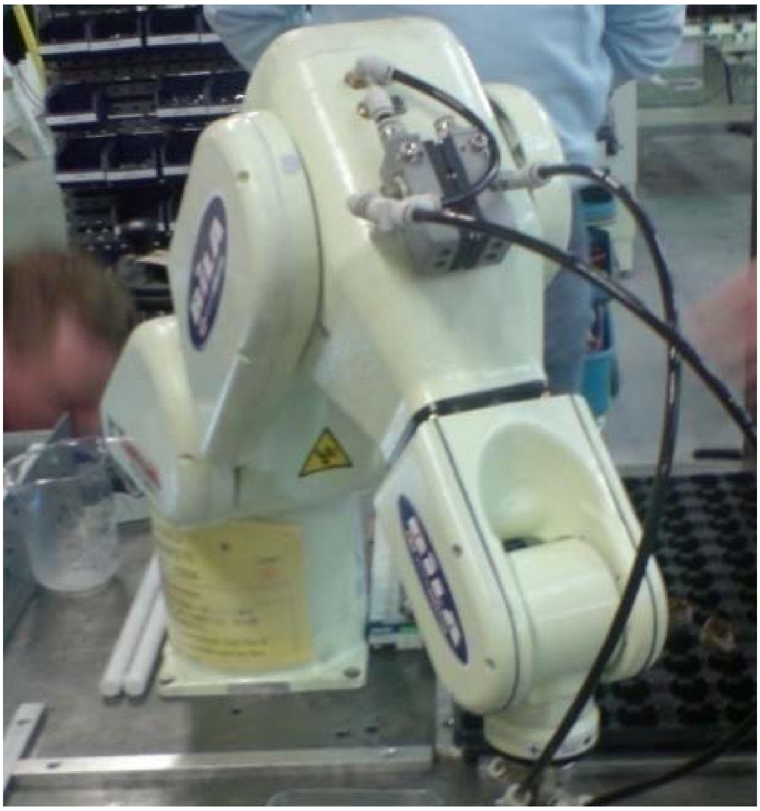

Fig. 4. Prototype of robot used in the project.

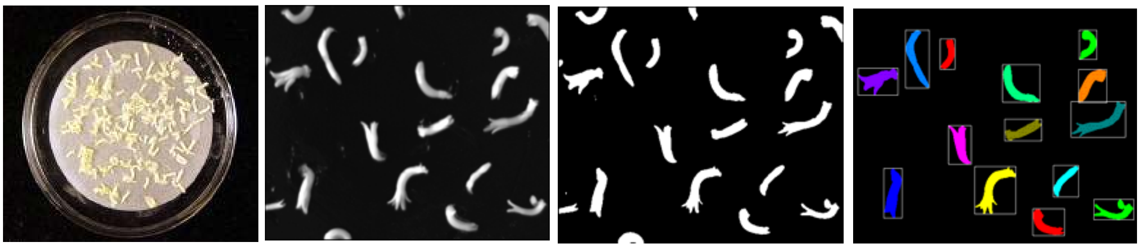

Fig. 5. Isolation and visualisation for quality assessment, sorting and orientation of 3-5 mm long mature embryos. The automated quality assessment is based on digitalized photos of mature embryos. Physical isolation of each mature embryo is required for visualisation and automated handling because embryos that are situated too close will be perceived as one structure by the software. The software can then distinguish different classes of embryos and can find the correct orientation, in respect to root and top. The software is trained to focus on classes of embryos that have been identified as high quality by an experienced technician. The first prototype can handle one embryo every 4 seconds.

B) Identification and transfer of germinated plants to soil (Fig. 6)

- Development of visualisation technology for identification of germinated plants with root.
- Development of handling technologies for transfer of plants from sterile growth medium to non sterile growth plugs.

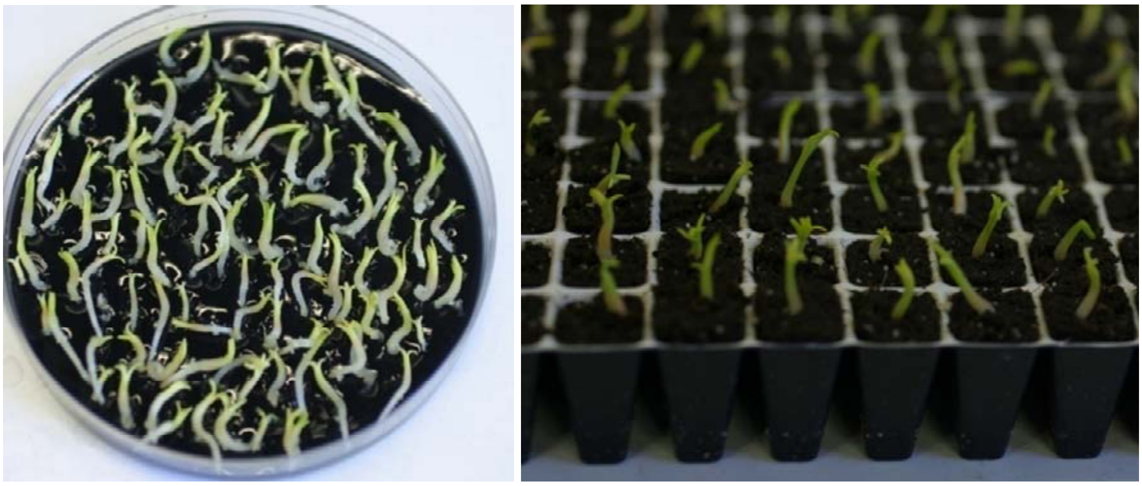

Fig. 6. Transfer of germinated embryos to growth plugs. The second step in the automated process is the transfer of rooted embryos from germination medium into growth plugs. The software identifies orientation and a grip point based on recognition of hypocotyle and root, and a handling device has been developed, which can handle the one plant every 4 seconds.

Conclusion

It has been possible to develop a prototype for automated handling of the two described processes in somatic embryogenesis. From a biological point of view, the most challenging point has been to develop the biological methods, so that they are suited for automated processing, e.g. by development of methods that ensures synchronised plant development during the different stages of somatic embryogenesis.

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Working Papers of the Finnish Forest Research Institute 114: 78–80

Possibilities for EU and Nordic research funding

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EU and Nordic research funding provide only limited possibilities for bottom-up research projects in which the research topics are defined by researcher and which have no limitations for the types of participating organisations or the place of performance. However, there are plenty of funding opportunities for pre-determined research themes, regional development projects, research supporting SMEs, researcher mobility, researcher training and research networking.

Research projects

EU's Seventh Framework Programme (FP7) includes ten thematic programmes. Each of the programmes publishes annually calls where the research topics are specified. Especially the programmes "Food, Agriculture and Fisheries, and Biotechnology" and "Environment" may include topics in the field of vegetative propagation.
(http://cordis.europa.eu/fp7/cooperation/home_en.html)

"Ideas" scheme of the EU's FP7 funds pioneering frontier research in any field of science. It provides funding for both starting and advanced top-level researchers. The research topics are defined by the applying researchers themselves (http://erc.europa.eu/).

SNS (Nordic Forest Research Co-operation Committee) funds research projects involving at least three Nordic countries. Estonia, Latvia, Lithuania and north-western Russia may also be included. SNS contribution is limited up to 15000 €/year/participating country.
(www.nordicforestresearch.org)

Development projects

EU's structural funds aim at enhancing economic and social cohesion; at strengthening regional development, competitiveness and employment; and at supporting the integration of border areas and larger cooperation areas. Projects must include strong development aspect and co-operation between different sectors such as academia, industry, authorities, and municipalities. Many of the programmes cover only a restricted geographical area. The programmes "Baltic Sea" (http://eu.baltic.net/), "Northern Periphery" (www.northernperiphery.net/) and "Interreg IVC" (www.interreg4c.net/) provide opportunities for Nordic-Baltic cooperation.

EU's Life+ programme funds best practice, demonstration and innovative projects that contribute to environmental protection or biodiversity issues. Projects can be either national or European. (http://ec.europa.eu/environment/life/)

Research projects involving small and medium-sized enterprises (SMEs)

EU's FP7 has a special programme for research for the benefit of SMEs and SME associations. It aims at strengthening the innovation capacity of small and medium-sized enterprises. At least 3 SMEs from 3 different countries and at least 2 research performers are required. (http://cordis.europa.eu/fp7/capacities/research-sme_en.html)

In addition, participation of SMEs is an asset in most of the other funding schemes in FP7 and structural funds.

Researcher mobility

Marie Curie Actions (People programme) of the EU's FP7 provides funding for researcher mobility. It grants fellowships for research in another European country or outside Europe. It also funds fellowships for third countries' researchers in Europe. International Staff Exchange Scheme grants financial support for researcher exchange between Europe and third countries. Industry Academia Partnerships and Pathways programme supports researcher mobility between private enterprises and research institutions.
(http://cordis.europa.eu/fp7/mariecurieactions/home_en.html)

Research infrastructures

EU's FP7 includes Infrastructure programme that funds new and existing infrastructures, such as large scale research installations, collections, special habitats, data-bases etc.
(http://cordis.europa.eu/fp7/capacities/research-infrastructures_en.html)

Research networking

European Science Foundation (www.esf.org) and SNS (Nordic Forest Research Co-operation Committee, www.program.forskningsradet.no/sns) fund workshops, conferences and research networking. In addition, COST (www.cost.esf.org) and NordForsk (www.nordforsk.org) fund research networking. Funding of SNS and NordForsk is restricted to Nordic and Baltic countries and North-Western Russia.

Preparatory projects

SNS (www.program.forskningsradet.no/sns) funds preparatory studies for larger projects and preparation of applications to EU. NordForsk has seed money for supporting excellent research at early stage (www.nordicforestresearch.org).

Researcher training

NordForsk funds research training courses, PhD supervisor courses and Nordic Networks of National Research Schools (www.nordforsk.org). Marie Curie Actions (People programme) of the EU's FP7 provides funding for research training programmes. It includes funding for both young researchers (doctoral students) and for experienced researchers for supervising and training as well as summer schools and workshops (http://cordis.europa.eu/fp7/mariecurieactions/itn_en.html).