IUFRO

Division 2 Joint Conference:
Low input breeding and genetic conservation of forest tree species

9-13 October 2006 Antalya Turkey

Proceedings

Preface

Small scale tree domestication, tree breeding and genetic conservation activities face different challenges compared to large and highly developed programs. There are various economical and logistical constraints as listed below, particularly in developing countries.

- In general, there is not sufficient financial support or land to develop rigorous and large-scale tree breeding programs for many forest tree species
- The scale (and cost) of the improvement program should be customized to the (financial and planting) capacities of the end users, or the cultivation range of the species.
- Most countries have to deal with multiple species
- Some countries have forest land with steep slopes and low fertility land. Progeny testing is costly and physically challenging.
- In most instances, conventional tree breeding is not appropriate.
- Conservation of genetic resources of minor species may require different strategy compared to widely used forest tree species

To address problems outlined above, an international meeting was held on October 9-13, 2006, in Belek, Antalya, Turkey. The topics included the following:

- Low-cost domestication, breeding and testing strategies
- Economic analysis of small scale forest tree breeding and gene conservations strategies in reducing the cost?
- Use of molecular markers and biotechnology in designing and implementing cost efficient breeding and gene conservation programs
- The role of in-vitro / vegetative propagation in design and implementation of cost efficient breeding programs, the role of clonal testing and selection
- Low-cost gene conservation strategies
- Tree selection, domestication and improvement for agroforestry.

This booklet contains the program and the abstracts of the presentations at the meeting. The full versions of the presentations will be available soon at the congress website, and will remain there for about six months (until June 30th 2007).
http://www.akdeniz.edu.tr/english/iufro/

The organizing committee hopes that the congress and its outcome will serve the expectations of the forestry community along this line.

Best wishes for all…

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Preface to Proceedings

With advances in digital technology, organizers are less and less inclined to print conference proceedings. Proceedings are often published online because of obvious advantages: It is hassle free. It does not cost. It is accessible easily. The downside of online publishing is that the site hosting the material can go away!

The local conference organizing committee will be hosting the conference proceedings and other materials on their web site until June 31st 2007 (http://www.akdeniz.edu.tr/english/iufro/). I will also put conference proceedings on my university web page (http://www4.ncsu.edu/~fisik/) as long as I keep my job! However, I suggest readers to print a copy of the proceedings for their records. I am going to follow that suggestion and print a copy for myself.

The proceedings are not peer reviewed. The abstracts and manuscripts were included in the proceedings as received, except some formatting. I strongly encourage readers to contact authors for details, perhaps for a copy of a recent manuscript on the same subject. If conferences still have a place, this is because they bring people together to interact and collaborate.

Organizing a conference is a lot of work. You soon realize that you have duged yourself into a deep hole!... but it is also a lot of fun. The best part is that you get to know a lot of people working in the same or similar discipline. I truly enjoyed the IUFRO Division 2 joint conference in Antalya, Turkey… I hope everybody liked it as much as I did.

Best wishes,

30th December, 2006

Fikret Isik

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ABSTRACT

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Gyuleva, V. 2006. Somatic Embryogenesis of Forest Trees in Bulgaria: Some Basic Problems & Future Prospects. *In* Proceedings of the IUFRO Division 2 Joint Conference: Low Input Breeding and Conservation of Forest Genetic Resources: Antalya, Turkey, 9-13 October 2006. *Edited by* Fikret Isik. p 86-92. Available from http://www.akdeniz.edu.tr/english/iufro/ [accessed 15 March 2007].

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The Next 30 Years

(Keynote Speech)

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Abstract

This presentation considers future needs for forests and wood under 3 scenarios: a smaller human population on Earth; a stable population of about 10 billion; and continued population growth. While there is great uncertainty about both the size of future human populations and their likely per-capita needs for wood, several plausible scenarios produce estimates for wood use by Earth's future peoples much greater than the recent 3.6 billion cubic meters being harvested per year.

Furthermore, there seems to be increasing widespread agreement on the desirability of allocating much increased area to wildlands, parks and reserves. Because of likely climate change, we will need to breed and/or manage secondary species not only for current conditions, but their needs for continued adaptation to changing conditions plus natural or assisted migration should be part of planning.

The trend toward shorter rotations is liable to reverse not only for primary species, but also for secondary species that contribute to wood harvest in managed stands.

A plea is made for people and organizations growing trees for harvested wood, and people conserving larger areas of Earth's forests in wildlands, parks and reserves, to seek the benefits of mutual agreements and cooperation in allocating forest areas to these uses.

Keywords: Climate change, Conservation, Ecosystem services, Rotation length, Wood production

Time

We will be discussing important ideas at this conference. I think it useful to consider what Earth will be like in the next several decades or centuries, as our ideas are translated to reality, or not.

Days and years are natural units. Millenia are not. The fact that our arbitrary millenium unit recently turned over, however, catalyzed some thinking about the future that may be useful. In the late 1990s, I participated in an exercise organized by FAO to consider what our forests, and our needs for wood, might be like five or more decades into the "next millenium".

On several occasions in the past, there have been predictions of a global wood shortage or even of wood famine. While such shortages have occurred locally, market forces and population dynamics have usually worked to bring wood supply into balance with demand, and Earth's peoples have not generally suffered long from severe wood shortages. This history may lead current decision-makers to be complacent with respect to future needs for forests. However, there may be needs in the near future for much more wood and other important forest services than present forests can provide.

While the FAO exercise focused on predicting conditions 50 or more years in the future, establishing the forests to satisfy those future needs should be accomplished many years before those needs become urgent. Thus, my title: “The Next 30 Years”, the time frame within which those future forests must already be present and growing.

The Needs for Forests and Wood in 2050 and Beyond -- Three Scenarios

A topic that is frequently ignored, particularly in the public statements of political figures, is the future sizes of human populations. The sizes of human populations, and the conditions that brought them to those sizes, greatly affect resource needs and availability.

First, let’s be clear that there is substantial uncertainty and disagreement about future human population sizes, and how they interact with resource availability and use. Rather than present all of the different projections considered in the FAO exercise and elsewhere, or pick my favorite, I’ll now present three contrasting but plausible projections and how they were derived. This should allow you to substitute your own numbers and predictions as you’re considering how your schemes might work in the future.

In 1900, there were about 1.5 billion people living on Earth. By mid-century, that total population grew to about 3 billion and by about 1999 it again doubled to 6 billion. *Scenario 1* suggests that human population growth will continue as in the 20th Century, again doubling twice to a population of about 24 billion living people on Earth in 2099.

Scenario 2 suggests that total human population size will soon be reduced to about 4 billion, a human population load at which some thoughtful people consider that Earth can provide a decent carrying capacity for most of those 4 billion people and for most of our fellow biota. This perhaps surprising scenario is based on the falling birth rates in places like Italy and Japan, and suggests that most or all other human populations will soon follow this trend.

Scenario 3 suggests that total population size will have stabilized in the range 9-to-10 billion by about 2050. It appears that two developments may be effective in achieving stabilization in those human cultures or political units with populations that are currently continuing to increase. One is the education and empowerment of women, apparently important but not further pursued in this presentation. The second is an increase in standards of living. This is of crucial importance to our considerations.

Earth’s recent total human population of 6 billion used about 3.6 billion cubic meters of wood per year. A projection of future needs for wood might just take current per-capita consumption of abut 0.6 m^3/yr and multiply it by projected population. But it’s not that simple.

As Jacques Cousteau suggested in his address to the 1992 Rio meeting on resource husbandry, most of the 24 billion people in *scenario 1* are likely to be living pretty much like rats. People living like rats don’t use much wood. Thus, if the population growth rate of the 21st Century continues as in the 20th Century, total wood use by 2099 is likely to be less than the current 3.6 billion m^3/yr., in large part because there probably wouldn’t be much forest left to use.

In *scenario 2*, if most of those projected 4 billion people can achieve standards of living pretty much like that of Americans today, with per-capita wood use near or greater than recent

American per-capita use of over 2 m^3/yr, that will require harvesting more than 8 billion m^3/yr. Where will that extra 5 or so billion m^3/yr come from?

Data from many countries indicate that, as standards of living have risen, birth rates have usually fallen but per-capita use of wood has increased. If the *scenario 3* stabilization at about 10 billion people on Earth is achieved in part by increased standards of living, including an average per-capita wood use of perhaps 1.5 m^3/yr, that requires about 15 billion m^3/yr of wood harvest, a huge increase.

Substitutes for Wood

Can other materials increasingly substitute for wood? During extraction and manufacture, wood requires less energy and emits less toxic chemicals and fossil carbon than any of the present alternatives. If energy becomes more abundant and less expensive, and inexhaustible sources of alternative raw materials are found, then per-capita needs for wood will be reduced by substituting these alternatives. This would probably result in the unintended consequence of a warmer more-toxic world. But a plausible prediction is for a future where energy becomes more expensive and non-renewable raw materials are less available. In that situation, wood will increasingly substitute for those other products and per-capita use of wood will be even higher, making the above demand estimates conservative (and Earth probably cooler and less toxic).

Natural Forests or Plantations?

With respect to forest allocation, I assert that we do not now have enough area in wildlands, parks and reserves, and that we should have a lot more of these in our future. I further assert that most of our forests managed extensively for some wood harvest suffer from, among other things, species-composition change, and highgrading within the more-valuable species.

Interestingly, the one and only thing those in the FAO exercise agreed on was that, in the near future, a greater percentage of our wood needs will be supplied from intensively managed plantations. In this short presentation I only note that, besides providing wood, many other important forest-ecosystem services will also be provided in varying degree by various kinds of plantations.

What About Climate Change?

Until recently, most planners and the general public assumed or accepted that the present climate is stable, and the future was planned for similar conditions. Today, planners have begun to comprehend that increased levels of greenhouse gases may result in general warming, with serious consequences for near-sea-level communities, and uncertain effects on most or all ecosystems globally. However, the possibility has been raised that Earth is in a general cooling phase being masked and offset by greenhouse-gas warming.

Repeated cycles of long glacial periods and much shorter interglacials have occurred during the past 2.6 million years, and we are currently a bit late for the beginning of the next glacial period. Whether some natural event has reversed the general cooling trend of the past 4,000 years and is now warming Earth is not certain. It is clear that anthropogenic inputs, such as extensive deforestation, wet-paddy rice culture, burning fossil fuels, cement manufacture, and vast areas paved or painted with dark materials, are keeping Earth warmer than it otherwise would have been. It is not clear whether or for how long such anthropogenic inputs can forestall or even prevent the next glacial period.

Both short-term warming and longer-term cooling need to be considered. It is clear that currently-extant forest-tree species have survived the repeated climate cycles of the past 2.6 million years. The boreal and temperate species have generally survived by both migrating and adapting to such things as different daylength cues. So, there seem to be three important questions to consider. (1) Will human modifications of the landscape, such as cities, farms and roads, impede migration of forest populations enough to cause many extirpations and even extinctions that would not have occurred in their absence? If the answer is "yes", perhaps we ethically should mitigate anthropogenic impediments to natural migration. (2) Will forest migrations need to be faster, more massive, and more effective than would occur naturally, in order to continue to support a human population of (say) 10 billion with wood and other forest products and services? If the second answer is even "maybe", we need to begin seriously thinking about how to responsibly expedite such forest migrations. (3) Will our breeding and management schemes maintain sufficient genetic variation to support adaptive evolution? If not, then what?

Rotation Length

In recent years, there has been a tendency for rotation lengths to be reduced, driven by financial pressures to recover capital quickly, and by needs for wood now. This may change.

Substitutions for steel, aluminum, bricks and cement will largely be for construction purposes, which generally require high quality if solid wood is used instead. This higher-quality solid wood is better produced on longer rotations. The recent increased use of reconstituted wood may reverse under higher energy costs, because reconstituted wood requires much more energy in its production than does solid wood. In a scenario proposed by Wink Sutton, most high-quality wood will be used first as solid wood, and then reclaimed for energy production in electricity-generation plants, or used as feedstock in a lignochemical industry, replacing petrochemicals both for electric generation and for many products such as plastics. (The demand for wood that is pulped, or burned for cooking and shelter-heating, will also probably hold up and intensify under higher worldwide energy costs. Such wood will probably be acquired as thinnings, as culls and tops during harvest, as whole trees from low-quality plantations and native forests, and as slabs and edges from sawmills.)

If indeed carbon credits become reliably available, they probably will largely be provided for two major forest-management options. (1) Carbon credits may reward increasing rotation lengths in established plantations and native forests. By financially rewarding increased rotation length, carbon credits will have some salutary serendipitous effects. One is a likely increase in plantation productivity, as the plantations will fully occupy the sites longer before harvest. A second is an increase in the quality and therefore value of the wood when harvested, from either plantations or native forests. (2) Carbon credits may also reward the establishment of new forests and plantations on land that is not now forested. This option will provide early cash-flow that may allow management to better afford early and mid-rotation activities, such as fertilization, thinning and pruning, that would increase both productivity and wood quality.

The Place of Secondary Species

Secondary species, perhaps by definition, seem unlikely to be widely deployed to plantations. There will be several good ideas presented in this conference about how to breed or otherwise aid their adaptations and increase their importance in serving both human needs and various ecosystem functions. Here, I'll just mention two strategies for their conservation, and some thoughts on their importance and management.

Ex-situ conservation is one option, using low-tech and/or high-tech biotechnologies, ranging from seed banks and clone banks through dedicated plantations and/or frozen tissue to DNA storage. Such options are variously expensive, with a need for active record-keeping and management. They are probably best used for species in danger of losing important populations or even of extinction. A problem with most of these options is that evolution, and particularly adaptation to changing conditions, is suspended.

In situ conservation, in adequate wildlands, parks and particularly in dedicated reserves, is an attractive option for so-called secondary species. Additionally, extensively managed forests will contain many secondary species, and some of those species will contribute to wood harvest from those forests. There are more secondary than primary forest-tree species and, at least for the near future, they will occupy much greater areas than will primary species in intensively managed plantations. Thus, their role in extensively managed forests, reserves, parks and wildlands will be very important with respect to the many ecosystem services such forests provide. These ecosystem services should be kept in mind as we consider how to breed, eugenically manage, or at least conserve these species. Given likely climate change, provisions for their natural migration or some forms of assisted migration are appropriate.

Conservation and Breeding for Use: Conflict or Cooperation?

We have people at this conference interested and effectively engaged in growing intensively managed plantations, usually of one or more so-called primary species, for wood production. Others are interested in conserving and/or breeding secondary species. Some of us are engaged in both, in programs including both primary and secondary species. Often in the recent past, we have not worked together very well on production and conservation and, too often, we have treated these as conflicting uses. We need a better strategy.

One strategy is exemplified by the New Zealand Forest Accord, in which people interested in conservation managed to find sufficient common ground with people interested in producing wood in intensive plantations. Rather than spending time and energy opposing each other in efforts to create wildlands, parks and reserves, or to establish new plantations, they agreed to rationally allocate lands to each of those uses. They support each other within this framework, or at minimum do not actively oppose each other as long as the accord is being honored. The thing missing from the New Zealand Forest Accord is large areas extensively managed for some wood production, the common situation in most of Earth's forests.

A more complete framework, not yet widely adopted, began to emerge in the last decade of the 20th Century in the U.S. state of Maine.

From the perspective of producing commodities such as wood, it is possible to conceptualize a "triad" of three types of land use: (1) plantation ecosystems where high levels of wood production are achieved; (2) variously modified ecosystems in which modest wood harvest occurs while various ecological values are carefully protected; and (3) protected ecosystems with virtually no wood harvest. Many environmentalists are reluctant to be advocates of plantation ecosystems, partly because of cases where much biodiversity has been lost during the conversion of natural forests to plantations. However, in some circumstances, it makes sense to switch wood production from extensive extraction in modified ecosystems to intensive production in plantation ecosystems, so that more land can be set aside in wildlands, parks and reserves.

The forests of Maine provide a good example. By 1990, only 2% of Maine's forest lands had been set aside as wildlands, parks and reserves, about 92% were extensively managed with various modest levels of wood harvest, and roughly 6% were plantations. Plantations in Maine produce about three times as much harvested wood per hectare per year as do Maine's extensively managed forests. Thus, for every hectare of forest switched from extensive management to intensively managed plantations, 2 hectares could be allocated to wildlands, parks and reserves with no net loss in wood harvested. It would be possible to increase Maine's forest wildlands, parks and reserves from 2% to 10% of forest area. To compensate for all of the foregone wood harvest on that reallocated 8% of extensively managed forest area, an additional 4% of extensively managed forests would be reallocated to intensive wood production in plantations, thus increasing the plantation area from about 6% to 10% with an overall reduction of extensively managed forests to 80% of Maine's forests. (By reallocating another 2% of Maine's extensively managed forests to intensive plantation forestry, Maine would no longer be a net importer of wood, but could once again become a net exporter to regions lacking sufficient forest.)

To conclude, I suggest that 21st Century intensive wood-producing organizations, and 21st Century conservation organizations, will be much more effective: (1) by finding issues on which they can agree; (2) by sensibly allocating or reallocating forest lands as in the Maine Triad; (3) by mutually supporting each other in these complementary endeavors; and (4) by avoiding the wastes of time and money that has been common in the intellectual and physical turf battles of the late 20th Century.

I suggest and hope we can find some bases for mutual support here in Antalya.

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Estimation of Genetic Parameters of *Pinus brutia* in the Aegean Region of Turkey

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Abstract

Three open pollinated progeny tests were established in low elevations of Aegean Region of Turkey. Each trial site contained 168 families from 8 populations and 6 control groups. Completely randomized block design with 4 tree row plot configuration was used in all trials. At the end of fourth growing season, tree height was measured. Breeding values were estimated by using BLUP method and genetic parameters were calculated. Statistical analysis revealed that differences among populations were statistically significant for height. Individual heritability ranged from 0.13 to 0.24 in four trials. The pooled heritability was 0.16 across the trials. Family heritabilities were moderate and ranged from 0.45 to 0.51. Type B genetic correlations were high among the sites and ranged between 0.70 and 1.00 suggesting that the population and family level genotype x environment interaction are negligible. The average height of plus trees selected was 2.6% higher than the control group (checklots). Selection of 30 trees with the highest breeding values would lead to 16.9% genetic gain compared to control group for the height.

Keywords: progeny trials, BLUP, heritability, genotype x environment interaction, breeding value, genetic gain

Breeding of Turkish red pine (*Pinus brutia* Ten.) in Turkey

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Abstract

Turkish red pine (*Pinus brutia* Ten.) is the most economically important tree species in Turkey. Breeding of Turkish red pine was started by the determination of the seed transfer regions in 1964. Since then, seed stands covering 12000 hectares have been selected to provide immediate seed source for reforestation. From the selected seed stands, 2170 plus trees have been selected phenotypically and grafted to seed orchards. The total area of seed orchards has reached to about 460 hectares by 2006.

The National Tree Breeding and Seed Production Program for Turkey was initiated in 1994 to progeny test plus trees. Five species were determined as target species in the Program. One of the main target species was Turkish red pine. Distribution of Turkish red pine was divided into 9 breeding zones. Intensive breeding activities (plus tree selection, seed orchard establishment and progeny testing) have been planned in 7 breeding zones. In other two zones, only seed stands and gene conservations forests were selected.

Progeny tests of Turkish red pine covering an area of about 90 hectares have been established in five geographic regions to test the plus trees. According to the program, survival (adaptability), growth rate and stem quality have been given emphasis. The early results from young (4 years old) progeny tests suggested that 14 to 24% genetic gain could be achieved compared to control group (6-10 seed stands). The results are encouraging to carry out an intensive breeding program. In addition to breeding, gene conservation activities have been implemented. Gene conservation forests covering 8000 hectares were designated to ensure future breeding activities.

Keywords: *Pinus brutia*, breeding, plus trees, seed orchard, progeny test, genetic parameter, genetic gain

Turkish red pine is distributed in Turkey, Syria, Palestine, Jordan, Iraq, Lebanon, Cyprus, Greece and Italy which are countries affected by Mediterranean climate. Widest distribution is in Turkey. It covers about 4 million ha that is 20% of total forest area of Turkey. Turkish red pine is the most important tree species in Turkey in terms of plantation and wood production. Both of them are about 40% of Turkey's. It is the fastest growing species within conifers therefore it has the shortest rotation period. Moreover seed production starts at age 3 and sufficient seed production starts at age 7 in seed orchards.

In Turkey, first organization of tree breeding studies were prepared by Faculty of Forestry, University of Istanbul. Breeding activities have been started by the establishment of "Forest Seed Research, Selection and Control Laboratory" at 1964. Seed stand selection, seed collection and storage, treatment of seeds prior to planting, seed export and management of seed orchards were done by this laboratory. Organization's current name is "Forest Tree Seeds and Tree Breeding Research Directorate (FTSTBRD)" and it has been involved in breeding of forest tree species including Turkish red pine since 1964. The institution is a key organization that works collectively with other governmental organizations in the area of forest tree breeding.

Breeding studies has been started on Turkish red pine together with other main species. Seed transfer regions for Turkish red pine with an elevation band of 550m was determined and rules of seed stand and plus tree selection revealed first at the year 1964. In 1972 seed transfer regions was revised and made narrower. Practical implementations on breeding were started. Selection of seed stands and plus trees from seed stands were followed by seed orchard establishment with grafted seedlings. First clonal seed orchard was established at 1976. In this context, breeding studies had been continued by phenotypical selection and seed orchard establishment until 1994. The National Tree Breeding and Seed Production Program (NTBSP) for Turkey was implemented at 1994. Five species were determined as target species in the Program. It was aimed to meet seed demand of 150.000 ha/year plantation by those species. One of the target species was Turkish red pine with about 50.000 ha/year plantation. Nine breeding zones were determined by considering elevation (at every 400m) and geographical regions. Intensive breeding activities (plus tree selection, seed orchard establishment and progeny testing) were planned in 7 zones. Other 2 zones were reserved for only seed stand and gene conservation forest selections (Figure 1). It was aimed to establish minimum 2 progeny tests in each of the 7 breeding zones. Survival (adaptability), growth rate and stem quality characteristics of Turkish red pine have been given the emphasis in the program.

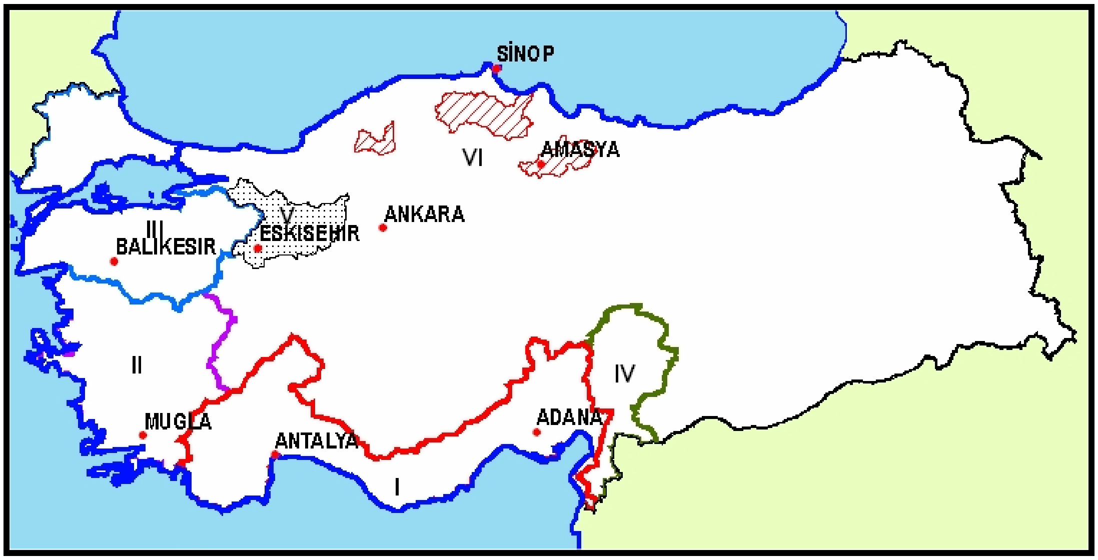

Figure 1. Turkish red pine breeding zone (I-IV, intensive breeding, V, gen conservation level VI, seed stands level)

One of the most important constituents of the program is progeny tests. In progeny tests it was aimed to determine breeding value of plus trees, to carry out rouging in existing seed orchards by considering breeding values of plus trees and to establish genotypic seed orchards containing the best 30 plus trees in the associated breeding zone. In addition, genetic parameters would be obtained in each breeding zone. First progeny tests were established at 1998. Up to now (2006), establishment of progeny tests have been completed in five breeding zones. Although plot arrangements were differed in some breeding zone, completely randomized block design was used in all progeny tests. Both plus trees in seed stands and clones in seed orchards were used in each breeding zone. Because of being plus trees in seed stand, unselected trees can contribute gene pool but in seed orchard only selected tees (clone) can contributed gene pool. Therefore, by considering the fact that gene frequencies could be different in this 2 groups, progeny tests were established as 2 series in each breeding zone. In each progeny test 6-10 seed stand was used as control.

Detailed information about established progeny tests is given in Table 1. Evaluation of measurements revealed that 14-24% genetic gain would be estimated by selection of the best 30 when compared to checklots (6-10 seed stands). Another important aspect of the program is gene conservation forests that are established to ensure future of breeding studies. The program proposed the presence of at least one gene conservation forest having at least 100 ha region in each breeding zone. Sampling of marginal distribution, selection of natural forests with the least treatment and constitution of different age classes were considered during the selection of gene conservation forests.

Table 1. Established progeny tests in breeding zone by 2006

Breding Zone	**Year of Establisment**	**Test number**	**Number of Plus trees/clone**
Mediterranean Low (0-400m)	1998	3	168
	1999	3	136
Aegean Low (0-400m)	2000	3	168
		3	25
Mediterranean Middle (401-800m)	2001	2	270
		2	74
Marmara Low (200-600m)	2002	3	158
		3	160
Aegean Middle (401-800m)	2005	3	324
Total		**25**	**1483**

Breeding of Turkish red pine has significant advantages. First, it is the most widespread species in Turkey. Since it is the most commonly used species in plantations, there is an opportunity to use improved material in large scale. Second, it is an early flowering species (3rd year) so breeding cycles will be shortened. Third, it is a fast growing species. It has the shortest rotation period within economically important species. This period can be reduced by breeding studies and so yield could be increased in unit time. Fourth, nursery practices and silviculture of the species are well-known. In addition, there are many genetic studies about the species.

There are also some difficulties on breeding of Turkish red pine. First, the species is vulnerable to forest fires. Therefore, breeding sites should be secured. Second, there is a lack of trained personnel. Since, there are limited numbers of breeders they have numerous responsibilities on breeding activities. Since there is no local trained personnel, all breeding activities are being controlled by FTSTBRD from Ankara. Third, there is no center to carry out activities such as grafting, controlled crossing, etc. Forth, it is difficult to find appropriate trial sites or locations due to topographic conditions in Turkey. Fifth, cost of protection of trial sites is high due to lack of public awareness.

Current situation in breeding of Turkish red pine in Turkey is quite satisfactory. Seed stands that covers 12000 ha have been selected to provide seed source for immediate seed demand. 2170 plus trees have been selected phenotypically and cloned by grafting into seed orchards. The total area of seed orchards has reached to about 460 ha by 2006. Progeny tests covering an area of about 90 ha have been established in 5 breeding zones to test the plus trees. In addition to breeding, gene conservation forests covering 8000 ha were designated to ensure future breeding activities.

Conservation and Breeding of Mediterranean Conifers

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Abstract

Conifers are keystone species of natural Mediterranean ecosystems. They represent valuable and often unique genetic resources which currently face both challenges and opportunities for their conservation and use. Major challenges include population decline and structure changes due to forest fragmentation, intensive forestry practices, wild fires, pests, diseases and especially climate change, that is expected to severely affect the Mediterranean Basin. The need for action to conserve, *in situ* and *ex situ,* the Mediterranean conifers' genetic diversity and especially of those species that could be more vulnerable due to their scattered and/or limited distribution is emphasized, while the role of tree improvement trials for conservation purposes and especially for the evaluation of conifers' adaptive potential is stressed. Marginal and disjunct populations may also be of high interest to both conservation and breeding as they have a higher probability of containing genetic resources adapted to stressful environments. Conservation of genetic diversity and tree breeding should be considered complementary rather than contradictory to each other.

The state of the art regarding conservation of genetic resources and breeding of Mediterranean conifer is reported. In particular, pertinent research is reviewed regarding the following species and their interspecific hybrids: *Abies alba, Abies cephallonica, Abies nebrodensis, Abies pinsapo, Cupressus sempervirens, Cedrus atlantica, Cedrus libani, Cedrus brevifolia, Pinus halepensis, Pinus brutia, Pinus nigra, Pinus sylvestris, Pinus leucodermis, Pinus peuce, Pinus pinaster and Taxus baccata.*

Keywords: tree improvement trials, genetic diversity, climate change.

Seedling Seed Orchards for Norway Spruce

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Abstract

The first seed orchards were established in Finland in the early 1950's. However, the large-scale establishment of seed orchards started in the late 1960's, and completed by the mid 1970's. The goal was to produce all the seed needed in nurseries and, in direct seeding from seed orchards. Currently there are 141 (2202 ha) Scots pine (*Pinus sylvestris* L.) and 25 (282 ha) Norway spruce (*Picea abies* (L.) Karst.) registered first generation seed orchards in Finland.

A new master plan for forest tree seed production was prepared in 2003-2004. The main objective of the new generation seed orchards is to increase genetic quality of seed. However, in Norway spruce, the number of clones, used in the first generation seed orchards is only 615 in the whole country. Therefore increasing the genetic gain of seed produced in the 1.5 -generation seed orchards is limited. To increase the number of clones, an additional Norway spruce plus trees were selected from 1990 to 2003. In total, about 1800 new plus trees were selected in the second selection to establish new first generation seeds orchards. However, grafting of the plus trees was not successful. The seed collected from the plus trees was used for further progeny testing started in 1995. The idea was to convert some of the progeny tests planned especially for seed production to seedling seed orchards.

In a new seed orchard program the aim is to establish 5 seedling seed orchards of an area of about 50 ha, and plant in these orchards about 400 plus tree progenies in 2004-2007. About 100 progenies are used in one seed orchard using the planting density of 4000-5000 seedlings/ha. Seedling seed orchards will be thinned at least twice before seed production phase, which will start 10-25 years after planting. The results of parallel progeny tests, as well as the results from the orchard itself are used in thinning. The costs of seedling seed orchards are only 50 % of the costs of grafted seed orchards (350 grafts/ha).

Keywords: *Picea abies*, seedling seed orchard, progeny test

Domestication of Forest Angiosperms Associated with Low Input Breeding Activities: Lessons of the Past – Challenges for the Future

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Abstract

Breeding of forest tree species is a rather recent human activity, extending to about one and a half centuries long. Over this time frame, forest tree breeding has been hindered by the necessity for high investment in time and space, long generation intervals, complicacies of a multitude of species' genetic and reproductive characteristics and evidently the presence of linkage equilibrium. Under these circumstances, breeding activities have been, in most cases, associated with low cost and low input. Nevertheless, significant success stories have emerged and the improved genetic material of economically important species (elite families, genotypes and clones) has multiplied wood production and improved the quantity and quality of other forestry products. Paradigms of such cases as they were experienced in Greece, involving different approaches, such as the classical provenance – progeny – selection depicted in the breeding schemes of multipurpose forest species (*Castanea sativa, Prunus avium, Junglans regia*), as well as the clonal approach reflected mainly in breeding *Populus*, *Platanus* and *Salix* will be reviewed. The experiences gained will be discussed in light of the contemporary status of forest tree breeding: need for immediate results under environmental stress and climatic change, low budget, and availability of a wealth of molecular genetic markers, DNA sequences and genomic tools. The notable initial genetic gains observed, owing by and large to the earliness of the domestication process, cannot be realized in the future unless under the frame of combined processes involving international cooperation in both classical and molecular breeding.

Keywords: breeding, domestication, multipurpose species, Fagaceae, Salicaceae

Ability of Peroxidase Patterns in Ecotype and Phenotype Separation of Wild Cherry and Mountain Ash

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Abstract

Biochemical markers like as Peroxidase enzymes are very useful in separation of ecotypes, phenotypes or genotypes because of theirs isoenzyme patterns diversity. In this research, twenty individuals of Wild cherry and Mountain ash was signed and sampled for enzymatic studies in two different forests and height from sea level (10 ind. per each elevation). Two years branches of the individuals (5 Ind. With Suitable morphology and 5 Ind. With unsuitable morphology per each elevation) were collected in the similar ecological conditions and winter. The quantity and quality activities were measured by spectrophotometer in 530 nm and PAGE method respectively during three years continuously. The results indicated that the individuals were separated in four classes for each species. The index isoenzymatic patterns of the individuals with suitable and unsuitable morphology and also two elevations were differed in two or three bands. It was also observed negative correlation between stem quality and quantity activity of Peroxidase. This research indicated valuable genetic diversity of Wild cherry and Mountain ash in Caspian's natural stands. In addition, it was established the role of Peroxidase enzyme as a tree's ecotype and phenotype indicator.

Key words: Isoenzyme, Peroxidase, PAGE, Spectrophotometer

Introduction

Wild cherry (Cerasus avium) and Mountain ash (Sorbus torminalis) are two most important species in natural forest ecosystem of north Iran aspect of fast-growing and wood quality for plantation and rehabilitation of destroyed stands. The several researches have proved that Peroxidase enzyme (POD) is the most important enzyme for reflecting natural or unnatural environmental stresses. Further this enzyme was used amply for genetic classification because of multiplicity of its anionic and cationic isozymes. This ability caused to study the enzymatic pattern alterations effect of height from sea level and also phenotype diversity (2, 3 and 4).

Material and Methods

Twenty individuals of Wild cherry and Mountain ash was signed and sampled for enzymatic studies in two different forests and height from sea level (10 individual per each elevation). Two years branches of the individuals (5 Individuals with Suitable morphology and 5 Individuals with unsuitable morphology per each elevation) were collected in the similar ecological conditions and winter. The quantity and quality activities of POD were measured by spectrophotometer in 530 nm and PAGE method respectively during three years continuously.

Results and Discussion

The results indicated that genetic diversity of both species is desirable because of their natural and unmanaged sites and embrace 8 classes for Wild cherry and 9 classes for Mountain ash (Figures 1 and 2).

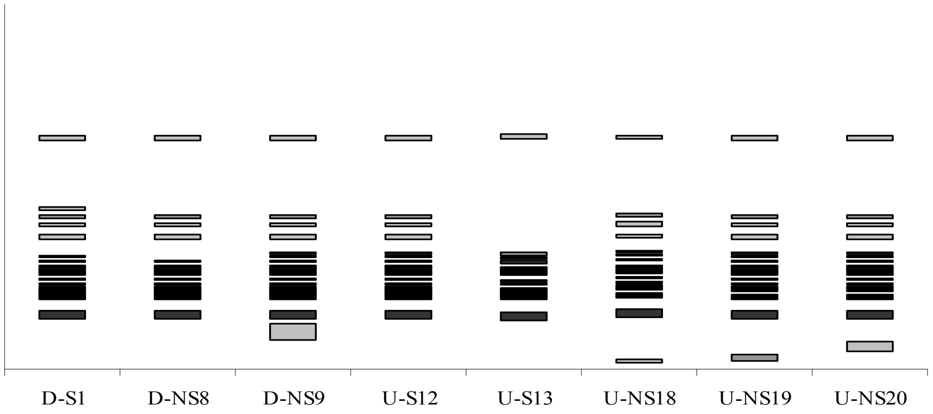

Figure 1: Genotypes and ecotypes diversity of *Cerasus avium*

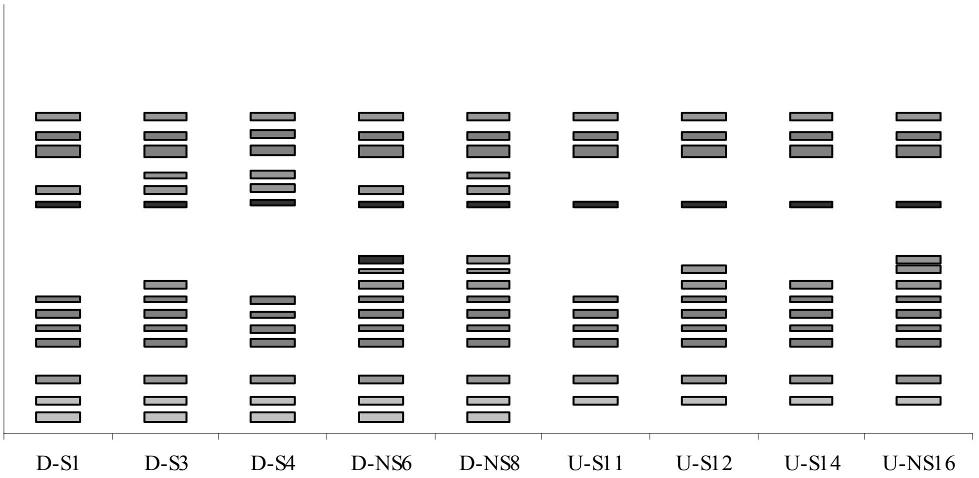

Figure 2: Genotypes and ecotypes diversity of *Sorbus torminalis*

Peroxidase isozyme patterns in two heights from sea level introduced some isozyme bands as h.s.l. index inclusive isozyme band no. 1 as up elevation index and isozyme bans no. 4 and 16 as down elevation for Wild cherry (Figure 3) and isozyme bands no. 1, 12 and 13 as both elevation index for Mountain ash (Figure 4).

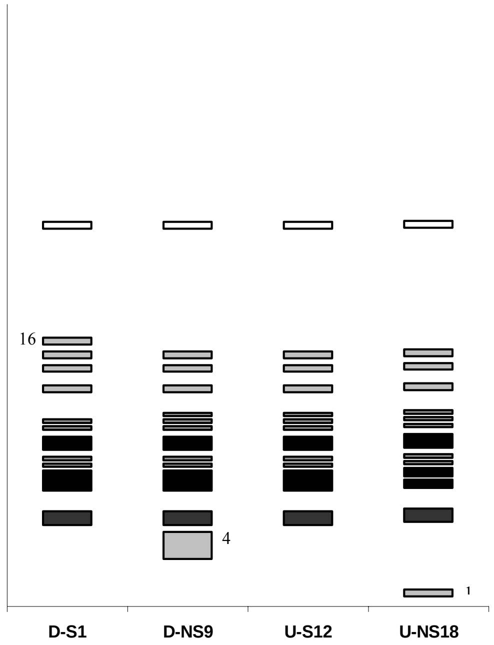

Figure 3: Camparison Wild cherry POD patterns between up and down elevations

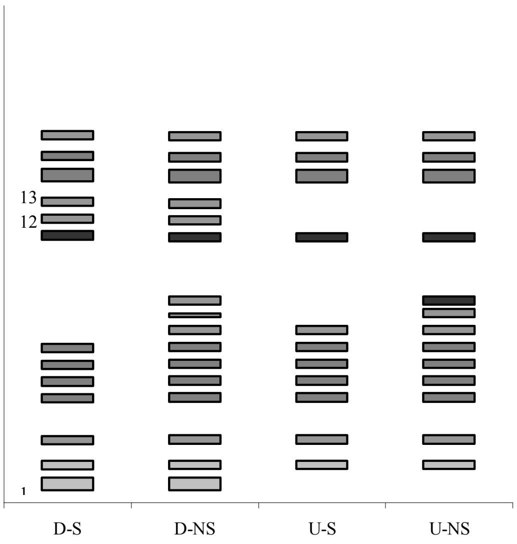

Figure 4: Camparison Mountain ash POD patterns between up and down elevations

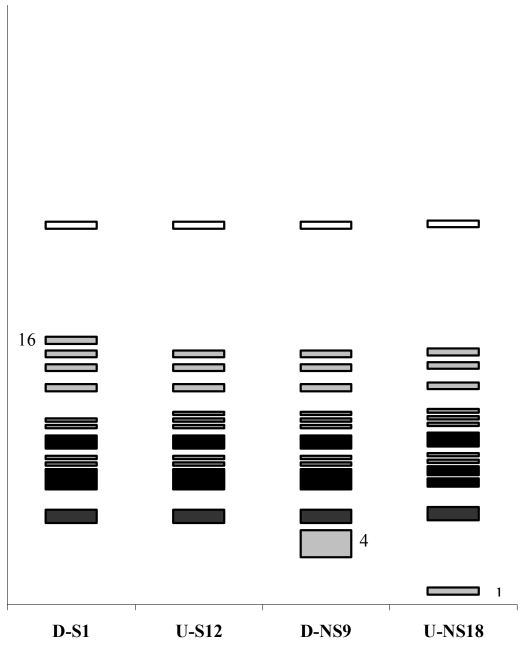

Figure 5: Camparison Wild cherry POD patterns between suitable and unsuitable phenotype

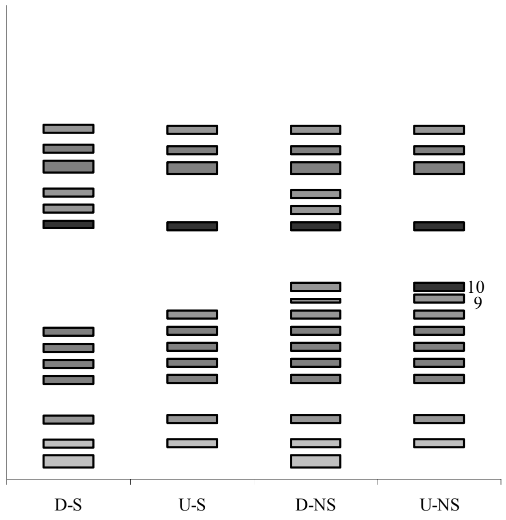

Figure 6: Camparison Mountain ash POD patterns between suitable and unsuitable phenotype

Furthermore, study of Peroxidase isozyme patterns between individuals with suitable and unsuitable phenotype aspect of stem quality in both species showed that unsuitable individuals had more isozyme bands than suitable ones and isozymes bands no. 1, 14 and 16 for Wild cherry and no. 9 and 10 for Mountain ash are as phenotype index (Figure 5 and 6).

Also, there was positive correlation (0.01 level) between quantitative activity of Peroxidase and stem quality degree (R=0.41). Cluster analysis of data including: presence or absence of bands, POD quantitative activity and stem quality degree by UPGMA and study of Esterase isozyme patterns confirmed two ecotype and two phenotype classes for both species (1).

Generally, these results showed that POD enzyme is useful for interspecific separation especially ecotype and phenotype diversity.

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New Method for Isolation of Resistant Eucalyptus against Salinity

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Abstract

Always we hear about destruction of environment especially forests because of increasing of population and demand for wood. Forestry with fast-growing species is a solution for conservation of natural resources. Eucalyptus is one of the species that has entered to Iran from 30 years ago. According to ecological requirements of the most Eucalyptus species (resistance against salinity, drought and high temperature) and the results of adaptation plans, the south of Iran is suitable habitat for plantation of Eucalyptus specially *Eucalyptus comaldulensis*. In this research, peroxidase as a biochemical marker was used for studying resistance against salinity because the enzymes are the most sensitive of indicators. Four individuals in Khansar and four in Carbandar region (Khozestan province) were selected for investigation of qualitative and quantitative alterations of Peroxidase. The samples were treated under different salinities and time periods. The leaves and branches did not show similar response against treatments so that the Peroxidase activity of leaves was more than branches in both regions. In addition, the samples of Carbandar branches had less alteration (Carbandar region had more salinity than Khansar region). In the resistant individuals' patterns, new isoenzymatic bands were appeared in the anodic region and also more Peroxidase activity. The results indicated that all individuals in Carbandar and one in Khansar region were the most resistant and can be used for multiplication. Poly acrylamid gel Electrophoresis (PAGE) and spectrophotometery methods were used for determining of qualitative and Quantitative alterations of Peroxidase, respectively.

Keywords: Isoenzyme, Peroxidase, PAGE, salinity

Fertility variation and gene diversity in a clonal seed orchard of *Pinus sylvestris*

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Abstract

Clonal differences in fertility (expressed as the number of female and male strobili) were determined for three years (2001, 2005 and 2006) in a clonal seed orchard of Scots pine (Pinus sylvestris). The seed orchard consisted of 29 clones (total of 1,265 ramets), and was established at northern part of Turkey (latitude 40°52'N, longitude 30°42'E, altitude 120m) in 1986. Grafts originated from plus trees selected in a stand and planted at spacing 7m x 7m.

The numbers of female and male strobili were counted for 25 clones. The number of ramets per clone varied from 11 to 58 (average 44 per clone). Reproductive output assessment was conducted for five ramets per clones randomly chosen and surveyed over the three years, avoiding ramets growing at the edges of the seed orchard. The number of female strobili for the sampled ramets was counted from a whole crown, but the number of male strobili was estimated by multiplying the average number of strobili per branch by the total number of strobili bearing branches.

Based on the reproductive output assessment, female and male fertility variation was estimated following the concept of sibling coefficient developed by Kang and Lindgren (1999). The female and male fertility variation was estimated by either relative contribution of clones or the coefficient of variation (CV) to strobilus production. Clonal fertility variation was estimated from the female and male fertility variation (Kang and El-Kassaby, 2002). Based on the observed clonal fertility variation, the status numbers (Ns, measure of genetic diversity) were calculated. The clones were similar fertile on both female and male sides. Pearson's correlation coefficients between female and male strobili production were positive for all studied years. Variation in female fertility was higher than that in male fertility in 2001, but it was opposite situation in 2006. Such variation was reflected on female and male parents' status numbers. Clonal fertility variation measured as the sibling coefficient was 1.10, 1.01 and 1.04 for the three years. The status numbers varied from 22.95 to 24.69 among the three studied years. On average (pooled), the status number (Ns) was more than 90% of census number (N), implying that most clones show near equal contribution. This even distribution of fertility by the clones is in contrast to the literature review by Kang et al (2001) who reported a typical value slightly above 2.0 (CV=100%) in mature clonal seed orchards.

Clones were grouped according to their female strobili production (i.e., seed production) into 5, 10, 15, 20 and 25 clones and then fertility and status number were estimated. This alternative might be seed as selective harvest from fertility clones. But, fertility variation was similar for female and male parents and total gametic gene pool for clone groups and years. Establishment and management of seed orchards were also discussed in the light of the results reported from this study.

Key words: Fertility variation, sibling coefficient, status number, flowering, Scots pine.

Introduction

Number of clones is one of the most important factor both economically (e.g. selection of plus trees, harvested seed orchard crop) and genetically (e.g. gene diversity of orchard crop). But, there were large differences among species and countries used on clone numbers in seed orchards. If many clones were used, genetic diversity would be high. But seed orchard cost would also be high. When a small number of clones are used, some rare allele in a base population may be lost in a seed orchard due to sampling effect (Bilir *et al*., 2004). For gene diversity of orchard crops, the number of clone may be more important than the equal number of ramet among clones (Kang, 2001). Many works were carried out on clone numbers (e.g., Kang *et al*., 2001; Bilir *et al*., 2004; Lindgren and Prescher, 2005) recently, while much previous discussion about inbreeding and genetic diversity in seed orchards on the number of clones required such as by Lindgren (1974), and Moran *et a*l. (1980). Establishment of seed orchard and estimation of seed demanding for the plantations are important stages in breeding programs. Gene diversity in seed crop used in plantations plays important role for sustainable forestry such as transmit of gene diversity from current generation to next. Estimation of gene diversity in seed orchard crop by fertility variation is a cheap method. Parental fertility of a tree is proportional contribution to the number of female and male gametes produced the tree (Gerigorius, 1989). Differences in gamete contribution among clones could be genetic (Eriksson *et al*., 1973), environmental (Hedergart, 1976), and management of orchard (Zobel and Talbert, 1984). Besides, years could be impact on flowering differences among clones (Lindgren *et al*., 1977). For instance, good seed years appear one time in two or three years (Ata, 1995).

The purpose of the study is to estimate fertility variation and gene diversity in seed orchard crop based on years and different census number of clones, and to discuss management and establishment of seed orchards.

Material and methods

Descriptions of seed orchard and data collection

The study was carried out in a clonal seed orchard of *P. sylvestris*. The seed orchard originated from plus trees, and was established at Sogutlu-Adapazari (latitude 40°52’N, longitude 30°42’E, altitude 120 m), northern part of Turkey in 1986. Grafts were planted at spacing 7 m x 7 m, and the seed orchard is composed of 29 clones (total of 1265 ramets) (Anonymous, 2001). Numbers of female and male strobili were counted in six ramets chosen randomly of each twenty-five clones in April of 2001, May of 2005 and 2006. The clones were grouped according to their female strobili production to 5, 10, 15, 20 and all (25).

Fertility variation

The female fertility (ψ_f) and male fertility (ψ_m) variation were estimated as (Kang and Lindgren, 1999):

$$\psi_f = N\sum_{i=1}^{N} f_i^2 = CV^2{}_f + 1 \,, \psi_m = N\sum_{i=1}^{N} m_i^2 = CV^2{}_m + 1 \qquad [1\text{-a\&b}]$$

where N is the census number, f_i is the female fertility of the i^{th} individual, m_i is the male fertility of the i^{th} individual and CV_f and CV_m are the coefficients of variation in female and male fertility among individuals, respectively.

Total fertility variation (Ψ) was estimated as (Kang, 2001):

$$\Psi = N\sum_{i=1}^{N} p^2{}_i = N\sum_{i=1}^{N}\left(\frac{f_i + m_i}{2}\right)^2 \qquad [2]$$

Where N is the census number, f_i and m_i are female and male fertility, respectively.

Status number and gene diversity

If the clones are unrelated and non-inbred, the status numbers of female ($N_{s(f)}$) and male parents ($N_{s(m)}$) are calculated as:

$$N_{s(f)} = \frac{1}{\sum_{i=1}^{N} p^2_{(f)}} \,, N_{s(m)} = \frac{1}{\sum_{i=1}^{N} p^2_{i_{(m)}}} \qquad [3\text{-a\&b}]$$

where $p_{i(f)}$ is the female fertility of the clone i, $p_{i(m)}$ is the male fertility of the clone i and N is the census number in the seed orchard. Here, fertility is estimated based on the strobilus assessment.

Status number on total fertility was calculated following as Kang (2001):

$$N_s = \frac{4N}{[\psi_f + \psi_m + 2 + 2r\sqrt{(\psi_f - 1)(\psi_m - 1)}\,]} \qquad [4]$$

where N is census numbers of clone, ψ_f and ψ_m are the fertility variation of female and male parents, and r is the correlation coefficient between female and male fertility.

Gene diversity (GD) was estimated based on total fertility (Ψ) and census number of clones (N) as:

$$GD = 1 - \frac{0.5\Psi}{N} \qquad [5]$$

Results and discussion

Strobili production

As seen from Table 1 and Figure 1, there were large differences for strobili production and coefficient of variation among years and clone numbers. 20% of the most productive clones produced 31% of total female strobili, 29% of male strobili in 2001, 23% of both female and male in 2005, 25% of female and 27% of male in 2006.

Table 1. Averages, coefficient of variation, correlation (*r*) of female and male strobili production for number of clones (N) and years.

	2001			2005			2006		
N	$\bar{x}_f$&$\bar{x}_m$	CV$_f$&CV$_m$	r*	$\bar{x}_f$&$\bar{x}_m$	CV$_f$&CV$_m$	r	$\bar{x}_f$&$\bar{x}_m$	CV$_f$&CV$_m$	r
5	299&737	0.05&0.14	0.73	293&804	0.12&0.17	0.83	404&738	0.06&0.10	0.78
10	263&631	0.15&0.15	0.75	277&784	0.10&0.10	0.79	378&641	0.09&0.09	0.80
15	243&585	0.19&0.24	0.73	270&771	0.09&0.11	0.77	359&607	0.11&0.18	0.79
20	217&553	0.28&0.24	0.72	264&756	0.09&0.10	0.75	343&600	0.13&0.19	0.72
all	193&522	0.38&0.27	0.69	253&734	0.12&0.12	0.78	324&561	0.17&0.24	0.74

*; statistically significant at 0.01 probability level.

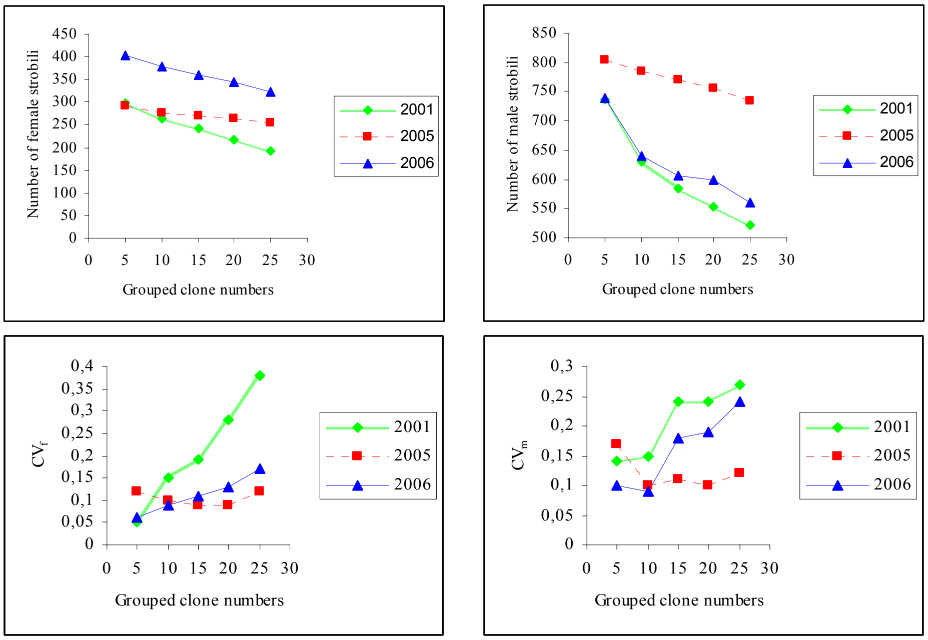

Figure 1. Averages, and coefficient of variation of female and male strobili.

Significant relation was found between female and male strobili in all year and clone number (*Table 1*). It was also reported by Jonsson *et al*. (1976), Bhumibhamon (1978), Burczyk and Chalupka (1997), and Bilir *et al*. (2002) in the species.

Large differences for average strobili and coefficient of variation among census number of clones and years were found (*Table 1*). But, there were significant relation between female and male strobili for both all year and clone numbers (*Table 1*).

Fertility variation, status number, relative status number and gene diversity

Although there were large differences among year and clone numbers for average strobili (Table 1 and Figure 1), parental and total fertility were similar (Table 2 and Figure 2). So, even year was one of the important factors on strobili production (e.g good seed year appears one time in two or three years in the species; Ata, 1995), it was not effective on fertility variation. Similar results were reported in natural stands of Brutian pine by Bilir et al. (2005). Maximum gene diversity of seed orchards crop can only be attained when all parents contribute equally to the gamete gene pool (Kang, 2001).

Table 2. Female and male fertility variation (ψf & ψm), total fertility variation (Ψ), status number (Ns) and relative status number (Nr) and gene diversity for number of clones (N).

	2001					2005					2006				
N	**5**	**10**	**15**	**20**	**all**	**5**	**10**	**15**	**20**	**all**	**5**	**10**	**15**	**20**	**all**
ψ_f	1.00	1.02	1.03	1.07	1.14	1.01	1.00	1.01	1.01	1.01	1.00	1.00	1.01	1.02	1.03
ψ_m	1.02	1.05	1.05	1.06	1.07	1.02	1.01	1.01	1.01	1.01	1.00	1.03	1.03	1.04	1.06
Ψ	1.01	1.03	1.04	1.06	1.09	1.02	1.01	1.01	1.01	1.01	1.00	1.02	1.02	1.02	1.04
$N_{s(f)}$	4.99	9.81	14.53	18.64	22.00	4.94	9.91	14.88	19.83	24.65	4.98	9.93	14.84	19.68	24.32
$N_{s(m)}$	4.92	9.57	14.25	18.95	23.34	4.89	9.87	14.84	19.79	24.67	4.96	9.71	14.56	19.30	23.70
N_s	4.98	9.73	14.45	18.93	22.95	4.92	9.90	14.88	19.83	24.69	4.98	9.85	14.73	19.59	24.13
$N_{r(f)}$	0.99	0.98	0.97	0.93	0.88	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.98	0.97
$N_{r(m)}$	0.98	0.96	0.95	0.95	0.93	0.98	0.99	0.99	0.99	0.99	0.99	0.97	0.97	0.97	0.95
N_r	0.99	0.97	0.96	0.95	0.92	0.98	0.99	0.99	0.99	0.99	0.99	0.98	0.98	0.98	0.97
GD	0.900	0.949	0.965	0.974	0.978	0.898	0.950	0.966	0.975	0.980	0.900	0.979	0.966	0.975	0.979

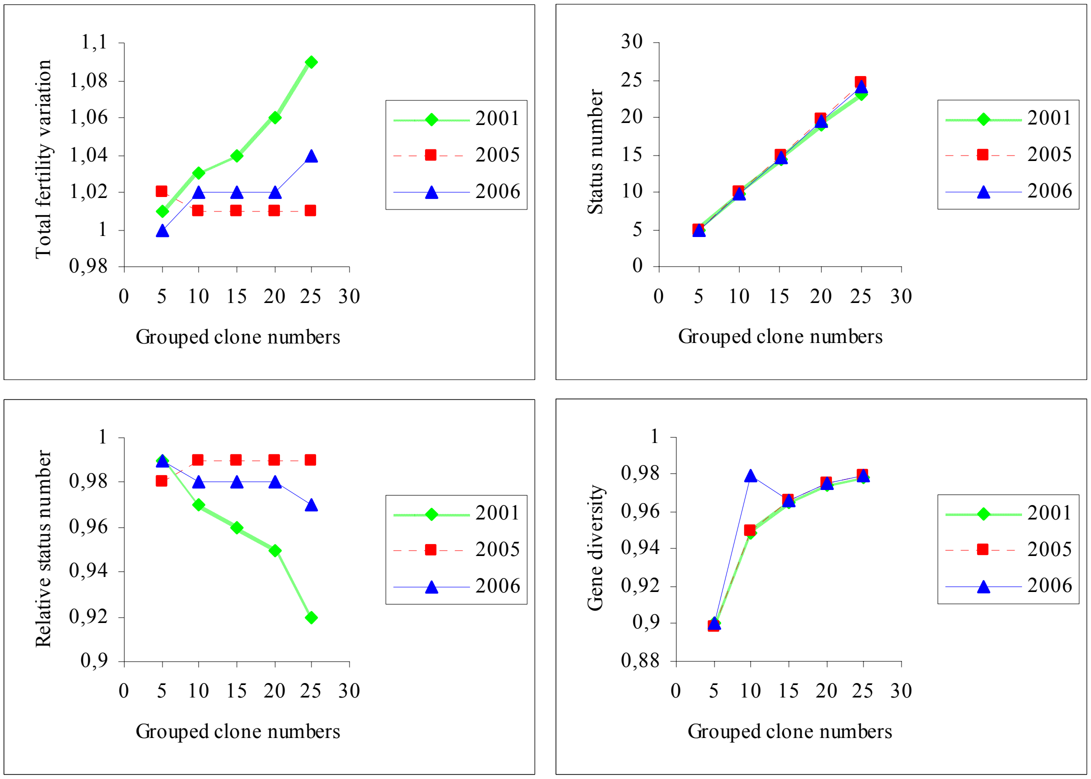

Figure 2. Total fertility variation, status number, relative status number and gene diversity.

It showed that less clones (e.g., 5-10 clones) could be used in establishment of seed orchards both economy and balance among clones (Table 2). Bilir et al. (2004) reported that even though the production of strobili is assumed to be proportional to the successful number of gametes, the number of 30 clones could hardly deliver entire gene diversity of the base population into the seed orchard. When used less clones, probably some rare alleles could also be missing when the base population transmits gene pool onto the seed orchard. But most breeding program does not focus mainly on the rare alleles that are not important for economic traits (Bilir et al., 2004). Maximum gene diversity of seed orchards crop can only be attained when all parents contribute equally to the gamete gene pool (Kang, 2001). The relation between gene diversity (GD) and fertility variation (Ψ), and census number of clones (N) can be showed as: GD = 1-(0.5 Ψ/N). It is also estimated based on status number (Ns) as: GD = 1- 1/(2*Ns) (Kang, 2003). When used many clones in establishment of seed orchard, fertility variation among and within clone will be high (e.g. CV) and then balance of fertility by seed orchard manager will be hard. Besides, effective number of clone can be low. So, fewer clones (e.g., 5-10 clones) should be used at establishment of seed orchard. it could be reduced to 20 or fewer clones after genetic rouging (Lindgren and Prescher, 2005).

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Thinning intensity effects analysis on rapid growth of planted Loblolly pine (*Pinus taeda* L.) stands using statistical MANOVA tests

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Abstract

Loblolly pine (*pinus taeda* L.) is one of the main exotic species, under commercial plantation in the north of Iran. These plantations are situated in the Caspian forests of Iran, which border the southern coast of Caspian Sea and cover an area of 1.9 million ha. For the present experiment, unthinned, light and moderately thinned stands were selected as three district populations. Data were collected from 180 trees in all, with different stand density. For basal area analysis, 93 plots were measured. Variables such as: diameter at breast height, total height, tree volume, bark thickness, basal area per ha, crown length and crown diameter were measured. Growth rate ratios of dbh in light and moderately thinned stands were 1.09 and 1.18 respectively. MAI of basal area growth rates in two thinned populations were 1.186 and 1.20. Moderate thinning increased the volume of stands by about 20.60% stands volume. This analysis indicated that moderate thinning drastically affects the basal area and the growth rate of volume. After thinning, dimensions of live crowns became greater and more branches became evident. Percentages of live crown ratios in three study populations were calculated as 41.23%, 43.87% and 44.52%. Growth rate interpretations were supported by statistical multivariate analysis of variance using Wilk's (Λ) and Hotelling's T_2^2 tests. The Wilk's test was carried out on mean vector of 6 variables in three study populations. The null-hypothesis (F_c =14.435> F_t =4.37) was rejected at the 0.01 level of significance. Thus, the results indicated that moderate thinning was significantly influenced the rapid growth rates of planted Loblolly pine stands.

Keywords: Caspian Sea, Loblolly pine, multivariate, growth rate, thinning

Provenance Variation for Growth Traits of *Pinus pinea* L. in Portugal

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Introduction

One of the most typical Mediterranean pines is the stone pine (*Pinus pinea* L.). In the Mediterranean area, the species is a valuable tree, useful for nut production, resin and wood production but is also used for shelter belts, leisure, or for erosion control.

A provenance trial was established in 1994, in two sites Tavira (Lat: 37º10'N Long: 7º36'W) and Sines (Lat. 38º01'N, Long: 8º42'W), and included provenances from Southern Europe (Portugal, Spain, Italy and Greece), Northern Africa (Morocco) and east coast of the Mediterranean Sea (Turkey and Israel) (Table1). Knowledge of the patterns of genetic variation is of fundamental importance to prevent problems of adaptation that may arise when seed sources are transferred into new environments (BEAULIEU, et al., 2004). The objective of this study was to compare and explain the variability of a stone pine (*Pinus pinea L.*) provenance trial established in Sines, based on growth traits at age two and thirteen.

Materials and Methods

This study was focused at "Perimetro Florestal de Sines" field trial. This site has Mediterranean climate with Atlantic influence, which is expressed by weak annual temperature oscillation, and high air humidity as a consequence of matinal fog incidence. The field design was installed according to a randomized complete block, consisting of six blocks, where each provenance was represented by 25 plants in a square plot, 3 x 4 m spacing. Total Height at age 2 and 13 (TH2 and TH13) and Diameter at Breast Height at age 13 (DBH13) were used in this study. All variables were measured in centimetres. The Least Square Means method (LSMEANS) in the GLM procedure of SAS (SAS Institute Inc. 1999) was used to predict provenances means for total height and diameter at breast height according to the following model:

$Y_{ijk} = \mu + b_i + p_j + bp_{ij} + e_{ijk}$ where,

μ = grand mean; b_i = effect of the i^{th} block, where (i = 1…b); p_j = effect of the j^{th} provenance where (j =1...p); bp_{ij} = the interaction of the i^{th} block and the j^{th} provenance; e_{ijk} = the interaction of the k^{th} tree in the i^{th} block and in the j^{th} provenance.

Individual tree values were used in the analyses for all traits. Data were analysed without border line inside each provenances plots (BURLEY, 1976).The Analyse of Variance (ANOVA) was performed using the procedure type III sums of square (SAS Institute Inc. 1999). The mean square for the provenance effect was tested for significance against the mean square for the interaction "block x provenances". In order to analyse significant differences, adjustment for Multiple Comparisons Test (Tukey-Kramer) was used. Variance components were obtained by using SAS VARCOMP Procedure (SAS Institute Inc. 1999) with the REML option. All effects were considered random.

Results and Discussion

Early-age survivals were generally high for all provenances. Survival percentages mean remained above 95 % for all provenances from ages two to thirteen. At age 13, the Moroccan provenance (M14) showed the best survival (100%), while the Greek (G3) and Spanish (S27) provenances were respectively the lowest among all studied (85%).

Table 1. List of provenances represented at "Perímetro Florestal de Sines", and their origin characteristics.

Code number	**Provenance name**	**Country**	**Latitude (N)**	**Longitude (W)**	**Elevation (m)**	**Annual rainfall (mm)**	**Temperature Min-Max(°C)**	**Average Temperature**
M1	Koudia Hamra	Morocco	35 11 00	6 10	25	594	6-31.6	
G2	Strophilia	Greece	38 08 00	21 22 00	20	748	1.3-37.2	17.8
G3	Mandraki	Greece	39 10 00	23 24 00	24	87,5	15.5-36	
It4	Cecina	Italy	43 45	29 00	2-4	847	-	
P5	Alc. Sal/Ervideira	Portugal	38 15	8 30 00	100	574	-	16.3
It6	Tomboli di Cecina	Italy	43 09	11 17 00	5	-	-	
M7	Air Grana	Morocco	35 16 50	5 53 15	970	960	4-36	
Is8	Monte Carmelo	Israel	32 45	35 00	400	600	-	
P9	Ponte de Lima	Portugal	41 46 00	8 36 00	300	1800	14	
P10	Vieira do Minho	Portugal	41 41	8 06 00	750-800	-	-	
P11	Amarante	Portugal	41 18 00	8 06 00	245	1250	-	
T12	Yatagan-katranci	Turkey	37 24	27 55 00	100	673.4*	16.3*	
Is13	Monte Carmelo	Israel	32 45	35 00	400	600	-	
M14	Cap Spartel	Morocco	35 47 16	5 53 11	200-300	887	9.6-26.8	
T15	Yalova	Turkey	48 32 38	29 22 49	500	-	-	
T16	Aydin-Karine	Turkey	37 46	27 23 00	450	693*	-	16.7*
T17	Kumluca	Turkey	36 17 45	30 20 02	5	-	-	
It18	Duna Felicia	Italy	42 02	12 27 00	5	-	-	
T19	Serik	Turkey	36 52 00	31 01 15	10	-	-	
M20	Dunes D' Adjir	Morocco	35 12 21	5 53 47	10	300	9.1-27.6	
T21	Çanakkale	Turkey	35 40 20	27 06 30	370	-	-	
P22	Viseu/Fig. Campo	Portugal	40 40 00	7 54 00	420	-	-	
S25	Andaluzia	Spain	36 20	65 00	631	-	-	
S26	Serra Morena	Spain	38 10	4 00	743	-	-	
S27	CordilheiraCentral	Spain	40 30	4 20 00	1007	-	-	

*FAO

The provenances effects explained 6.2% and 9.4% of total variability for TH2 and TH13 respectively. ANOVA showed that the effects of provenances, blocs and interaction were significant for TH2 and TH13 (Table 2).

Table 2. Levels of significance for each factor and variable (*TH2, TH13 and, DBH13)*.

	Anova levels of significance			Components of variance (%)			
	prov	block	prov x block	prov	block	prov x block	error
TH 2	0.0211	0.0001	0.0001	3.99	6.2	20.26	69.55
TH 13	0.0292	0.0001	0.0001	5.18	9.45	37.08	48.3
DBH 13	0.1209	0.0001	0.0001	2.48	12.05	29.72	55.74

Note: a) Level of Significance p > 0.05 – not significant; p ≤ 0.05 – significant; p ≤ 0.01 – high significant; p ≤ 0.001 – highly significant, b) in provenance test of hypotheses using type III Mean Square for blockxprov as an Error term.

Regarding DBH13, no significant differences were found for provenance effects due to the interaction amount (used as an error term). Table 3 provides the ranking of *Pinus pinea* L. provenances with Tukey-Kramer test at age 2 and 13 for TH and for DBH.

Table 3. Ranking of *Pinus pinea* L. provenances (ls-means) with multiple comparisons adjustment test (Tukey-kramer) at age 2 and 13 for Total height (TH) and for Diameter at Breast Height (DBH)

Code number	**DBH13 (cm)**	**Code number**	**TH2 (cm)**	**Code number**	**TH13 (cm)**
IT18	9.93	T16	31.68	It18	364.47
Is13	9.12	It4	31.53	Is13	340.28
It4	8.80	IT18	31.16	M1	335.61
E27	8.64	M1	30.87	It4	330.91
P10	8.60	M20	29.27	P11	319.65
M1	8.43	T21	29.00	P10	317.43
T15	8.13	P5	28.97	T15	312.54
E25	8.12	P10	28.63	E27	310.92
It6	8.02	P22	27.93	E26	309.67
M20	7.96	Is13	27.72	It6	305.58
T21	7.96	T15	27.61	M20	304.81
P11	7.92	E27	27.29	E25	299.56
E26	7.92	It6	27.20	M7	297.17
P9	7.81	P11	27.10	T21	292.59
G2	7.72	E26	26.85	G2	292.52
M7	7.10	T19	26.85	P9	288.43
P5	7.09	T17	26.58	Is8	284.43
Is8	7.01	P9	26.50	P5	279.23
T17	6.99	E25	26.43	T17	275.49
G3	6.64	M14	26.28	T19	272.25
T12	6.58	G3	25.48	P22	262.53
T19	6.53	M7	24.09	M14	258.46
P22	6.44	T12	23.51	T12	258.46
M14	6.19	Is8	22.84	T16	251.71
T16	6.14	G2	22.29	G3	244.86
Total mean	**27.35**		**27.35**		**296.38**

Means connected with a line do not differ significantly (Tukey's HSD test, $P = 0.05$).

Tukey-Kramer test revealed that five out of the 25 provenances analysed were very close, constituting the best group. At age 2, the best provenance was from Turkey (T16), but at age 13, Italian provenance (It18) appeared in the top of the ranking, while the worst provenance was from Greece (G3). The comparison of these two years showed that three provenances (It18, M1, It4) remainded among the best group. It seems these three provenances are stable for growth traits. Despite the economic value of *Pinus pinea* is mainly based on nut production, it seems that growth total height might also be an indicator for the adaptability of the species in this field site. The most stable provenances allow obtaining potential gains through the selection of the best trees among these best provenances. These trees might be included in a Portuguese *Pinus pinea* L. improvement program.

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The Effect of Management and Breeding Activities on the Established Anatolian Black Pine and Turkish Red Pine Forests

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Abstract

Turkish red pine (*Pinus brutia* Ten.) and Anatolian black pine (*Pinus nigra* subsp. *pallasiana*) are naturally distributed in Turkey and they are two economically important forest tree species in the country, listed as priority species in "National Tree Breeding and Seed Production Program for Turkey". Until tree breeding program became operational, the main seed sources for forestry activities were seed stands. With this study, it is aimed to assess how effectively the genetic diversity from natural populations (seed stands) is transferred to plantations. For this purpose, genetic diversity of 12 Anatolian black pine and 18 Turkish red pine seed sources were sampled and studied by RAPD markers. The seed sources were sampled at three different categories (seed stands (SS), seed orchards (SO) and plantations (P)) with the restriction of seed sources being originated from the same geographic location. Genetic diversity parameters were comparatively estimated among seed sources. Based on diversity parameters, for Anatolian black pine, there was no significant reduction in amount of genetic diversity of plantations (H_e for SS=0.39; H_e for SO=0.36, H_e for P=0.33) though there was slight reduction in proportion of polymorphic loci of plantation (SS=%97 vs. P=%89). For Turkish red pine, similar pattern was observed in genetic diversity (H_e for SS=0.24; H_e for SO=0.23, H_e for P=0.21) and polymorphic loci (SS=%62 vs. P=%57), but there was significantly lower genetic diversity in this species compared to Anatolian black pine. The results of this study have shown that the loss of genetic variation through management and breeding activities does not appear to have taken place in any of the managed forests for Anatolian black pine and Turkish red pine

Key words: *Pinus brutia*, *Pinus nigra*, seed sources, genetic diversity, RAPD markers

Assessment of the influence of domestication of Oregano, produced in Köprülü Kanyon National Park, on socio-economic conditions of forest villages

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Abstract

Köprülü Kanyon national park was declared on 12 December 1973 by Ministry of environment and Forest of Turkey. Total size of the park is 36 614 hec. It takes place in C_3 square of floristic grid system developed by Davis for Turkish flora in 1965. The park is situated in Mediterranean Region in terms of phytogeography. The elevation above sea level changes from 130 m (from Beşkonak village) to 2505 m (to Dernek Hill of Bozburun Mountain). The villages in Köprülü Kanyon can geographically be grouped in two . The ones situated down stream of Köprü River (the site is named by this river) and the others situated upland of the river basin.

The six of Oregano species exist in Mediteranean region are found in the Park, respectively *Origanum miniflorum, Origanum onites, Thymus leucotrichus Jalas., Thymus longicaulis C. Presl. Subsp. Chaubardii, Thymus revolutus Celak, Thymus sipyleus Boiss.* All the six species are harvested in the villages of the park, namely Altınkaya, Ballıbucak, Çaltepe, Değirmenözü and Beydilli. The production is approximately 350-400 tone/year. However, the conservation of biodiversity is a neccessity due to the conservation statue of the park. Therefore, the species harvested should be domesticated and then cultivated in suitable irrigated or non-irrigated agricultural areas in the sake of biodiversity.

In the study, the past and present and the future of Oregano production , one of the non wood products in Köprülü Kanyon National Park, are investigated. Besides, the study reveals investigations into the domestication of wild Oregano and its production and its past and present influences on socio-economic condition of the villages and its possible future influences.

Key words: Non-wood forest products, Oregano (origanum sp.), socio-economic structure, domestication

Effect of the Super Absorbent on Development of *Paulownia elongata* Seedlings

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Abstract

This paper presents the results of the effect of the super absorbent on the development of *Paulownia elongata* seedlings (fast-growing broadleaf species originating from China). During the first growing season (the most important growing period) lasting for about 4.5 months, the seedlings need a greater quantity of water. The bio-degradable super absorbent based on starch polymer absorbs and retains a great quantity of water, several tens of times more than its weight, and makes it available to the plant when it is necessary.

The seedlings of *P. elongata* were produced from seeds in the greenhouse of the Faculty of Forestry in Belgrade, after which they were transplanted to the sample plot. Based on morphological study, it was concluded that height and diameter increment, as well as leaf area, were several times greater than those of the control seedlings.

Key words: Paulovnija, super absorbent, height and diameter increment

Morpho-anatomic Characters of the Leaves of Yellow-leaf and Red-leaf Beech cultivars

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Abstract

This paper presents the results of the study of mopho-anatomic characters (stomatal dimensions and number) of the leaves of two ornamental cultivars of beech: yellow-leaved *Fagus silvatica* L. 'Luteofolia' (= *F. silvatica* L. var. *lutefolia* Tosic) and red-leaf *Fagus* silvatica L. 'Atropunicea' (= *F. silvatica* f. *atropurpurea* Kirchr.), as well as the typical variety of beech (control). *F.s.* var. *luteofolia* Tosic, was identified in 2005 near Kotor Varoš in the Republic of Srpska (Bosnia and Herzegovina) and it is the second finding of yellow-leaf beech with the typical habit in Europe, and *F.s*. f. *atropurpurea* has been known since 1680 in Switzerland (Krüssman, 1960). Based on the *Fagus moesiaca* (K. Maly) Czecz. var. *aurea* Obrad. (=*F.silvatica* var. *zlatia* Späth) found in 1890 on Vlasina near Vranje in Serbia, the well-known cultivar *F.s.*'Zlatia' (Späth) was produced in Berlin. In this study, the leaves from the vegetative copies of the ornamental cultivars were used (F. s. 'Luteofolia' - 5 trees and F.s. 'Aropunicea' - 2 trees) and from one beech tree *F. silvatica* L. of seed origin. Based on the analysis of morphometric measurements it was concluded that there are differences between the ornamental cultivars and the common beech, both in the size of leaves and in the size and number of stomata per unit area.

Key words: anatomic characteristics, yellow-leaf beech, red-leaf beech

Introduction

The spontaneous mutants of forest trees have for a long time been noticed and readily used as the initial material in the production of ornamental cultivars for horticulture (weeping and pyramidal forms, forms with different leaf colours, etc.). Especially attractive are the mutants with an unusual leaf colour ("golden" pines, yellow yew, yellow beech and red beech, red hazel, etc.), because they make a strong contrast to the green surroundings.

The results of the study of morpho-anatomic characters of the leaves of two ornamental beech cultivars are presented: yellow-leaf *Fagus silvatica* L. 'Luteofolia' (= *F. silvatica* L. var. *lutefolia* Tosic) and red-leaf *Fagus* silvatica L. 'Atropunicea' (= *F. silvatica* f. *atropurpurea* Kirchr.), as well as the typical variety of beech (control). *Fagus silvatica* L. var. *lutefolia* Tosic was found in 2005 near Kotor Varoš in the Republic of Srpska, and it is the second finding of such beech in Europe. The first yellow-leaf beech was found in 1890 on Vlasina in Southeast Serbia and it was used for the creation of the famous cultivar *F. silvatica* L. 'Zlatia' L. Späth, which decorates the European parks, gardens and arboreta. For a long time, the tree on Vlasina was forgotten by the professionals, and even it was unknown in our professional literature, and consequently in Serbia and in the former Yugoslavia its valuable characteristics were not exploited.

Material and Method

The leaves were taken from the vegetative copies of *Fagus silvatica* L. var. *lutefolia* Tosic, *F. silvatica* L. var. *atropunicea* West. and from the typical variety of *F. silvatica* L. of seedling origin (for control), which grow in the nursery of ornamental plants Tamaris Company d.o.o. in Banjaluka (Republic of Srpska), i.e. in the identical environmental conditions.

Fifty leaves were taken from each tree for the morpho-anatomic research. Their leaf length, leaf width, and stomatal sizes (width and length) were measured, as well as the number of stomata per unit area (mm^2). The number and size of stomata were measured on the lower epidermis of the leaf. The number and sizes of stomata were determined by the method «collodion process» (Volf, 1950). The values obtained for stomatal sizes were processed by computer programme «Statistika 6.0». The statistical justification of differences between the mean values of the anatomic characteristics of the leaves was calculated by Student t-test, probability 95% (Isajev, V., Šijačić-Nikolić, M. 2003).

Results and Discussion

A beech tree with yellow leaves aged about 250-300 years (from which the copies were obtained by heterovegetative method of reproduction) is a natural rarity, significant not only for the Republic of Srpska in which it grows, but also for Europe, because it is unique by its extraordinary morphological-anatomical, physiological and aesthetic characteristics (Fig. 1.).

Table 1. Statistical parameters of beech leaf dimensions

CULTIVAR	Measure (cm)	$\xi \pm S_{\xi}$	$S \pm S_{\xi}$	$V \pm Sv$
F. silvatica L. 'Luteofolia'	Length	83.08 ± 2.26	11.31 ± 1.60	13.61 ± 1.93
	Width	57.12 ± 1.60	7.98 ± 1.13	13.97 ± 1.98
F. silvatica L. 'Atropunicea'	Length	80.12 ± 2.16	10.79 ± 1.53	13.46 ± 1.90
	Width	53.16 ± 1.71	8.54 ± 1.21	16.06 ± 2.27
F. silvatica L. (Control)	Length	81.84 ± 1.67	8.36 ± 1.18	10.21 ± 1.44
	Width	47.08 ± 0.79	3.94 ± 0.56	8.36 ± 1.18

The copies of beech trees with yellow leaves (Fig. 2) differ from red-leaf beech and the typical beech by the colour of the leaves, dimensions of the leaves, as well as by the size and number of stomata per unit area. The leaves of yellow beech are very yellow and lustrous during the growth season, while the leaves of red-leaf beech change their colour during the vegetation period, from blood-red in the spring and in the first half of summer, to dark brown with a slight admixture of green colour during the autumn, which makes them a true ornament to the green surroundings (Fig.3).

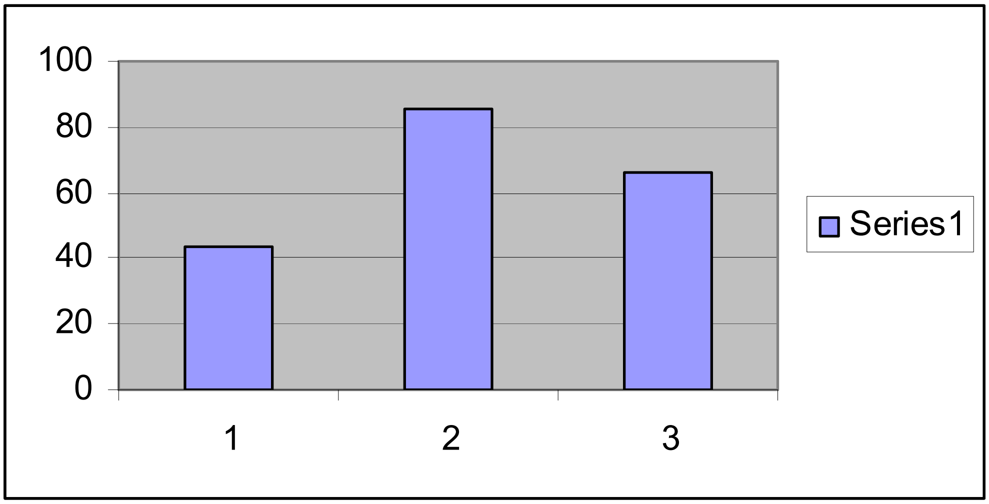
Figure 1: Yellow beech (Stem circumference 100 cm above ground is 408 cm!)

Figure 2: Yellow beech vegetative copies (grafts)

Figure 3: Red beech (F. silvatica L.'Atropunicea')

Based on the statistical parameters, the longest and the widest leaves occur on yellow beech (Table 1). Graphic 2 shows the number of stomata per mm² and it can be concluded that the red beech cultivar is statistically significantly different, it has a greater number of stomata (ξ =85.33) on the leaf underside compared to the yellow beech cultivar and common beech. A great number of stomata on the red-beech leaf underside indicates the development of the photosynthetically active tissue, which performs transpiration through the stomata. There are also statistically significant differences in the sizes of stomata. The smallest stomatal length and width was measured on red beech, which also confirms that the leaves with smaller stomata have a larger transpiration area (Table 3).

Graphic 2. Number of stomata on the leaf underside



1. *Fagus silvatica* L. 'Luteofolia'
2. *Fagus silvatica* L. 'Atropunicea'
3. *Fagus silvatica* L

Table 3. Length and width of stomata on the leaf underside

Length (µm)					
CULTIVAR	Mean value	Standard deviation	t - value		
			1	2	3
1. *F.silvatica* L 'Luteofolia'	14.88	1.39	-		
2. *F. silvatica* L.'Atropunicea'	9.56	1.25	14.85*	10.85*	-5.85*
3. *F. silvatica* L. (control)	11.34	1.32	-9.70*	-4.76*	-

Width (µm)					
CULTIVAR	Mean value	Standard deviation	t - value		
			1	2	3
1. *F. silvatica* L 'Luteofolia'	6.76	0.26	-		
2. *F. silvatica* L. 'Atropunicea'	5.74	0.90	-6.35*	-2.81*	17.16*
3. *F. silvatica* L. (control)	5.03	0.97	-9.08*	-6.24	-

Conclusions

Based on the results of the study of morphological and anatomical characters of the leaves, which are undoubtedly under genetic control, it was concluded that beech with yellow leaves *F. silvatica* L. var. *luteofolia* Tosic, by its characteristics, is significantly different from red-leaf beech *F. silvatica* L. var. *atropunicea* West., as well as from the common beech *F. silvatica* (Točić, 2005, 2006).

Thanks to the attractive colour of their leaves, the cultivars of both beech varieties will have a wide use in horticulture.

The study results of anatomic structure suggest that, during the application of ornamental cultivars of the study varieties in the town landscaping and landscape management, attention should be paid to site conditions, especially to soil moisture and air humidity. Based on the size and number of its stomata per mm², the red-leaf beech can be planted on the moister sites compared to both yellow beech and common beech.

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Ex situ Conservation of Forest Trees: Effective Strategy or Overstated Promise?

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Extended Abstract

Ex situ conservation of forest trees is meant to be a complimentary approach to *in situ* protection of local gene pools. Unfortunately, *in situ* conservation programs for some threatened tree species have never materialized, or if developed, have not lasted long, and the last remnants of germplasm accessions often rest in *ex situ* field plantings.

Ex situ conservation efforts are characterized by their own set of challenges. First, long-term *ex situ* conservation can be very expensive. Second, *ex situ* field plantings must have ecological importance or exhibit potential economic return to the receptor agency or interest in long-term protection of the tree species quickly wanes. Third, there is a growing reluctance of some countries to donate plant material for *ex situ* conservation purposes. Fourth, if genetic material from conservation collection in natural stands can be obtained in the donor country, plant quarantine restrictions in many receptor countries have now become so strict that legal transport of seeds for the establishment of field plantings for *ex-situ* conservation has become nearly impossible. Fifth, if *ex situ* conservation plantings are established successfully, generally long-term strategies are not in place to manage the genetic material once the trees have reached biological maturity. Ultimately, the question to be asked is, “Is the movement of tree germplasm from a location where it is threatened to a location where it is more protected an effective conservation strategy in the 21st century?”

In the last 26 years, Camcore, North Carolina State University (an International Tree Conservation and Domestication Program) has collected research amounts of seeds from 40 pine and broadleaf tree species and 11,500 mothers trees in approximately 470 native populations and have distributed these to members in 15 countries. For many of its species, hundreds of thousands of trees represent gene pools that have been planted across a myriad of climates and landscapes.

Many of the original Camcore *ex situ* genetic trials and conservation areas are approaching 20 years of age. Camcore’s goal is to conserve genetic material at the provenance level for populations it has classified in natural stands as vulnerable to critically endangered. Each of the 22 active members has been assigned specific provenances to protect for the long-term based on suitable climatic matches between donor and receptor countries and equally shared workload.

Field trials are thinned (genetically) beginning at about 10 years of age for tropical and subtropical pines to convert them to conservation banks or parks over time. Family pedigrees are maintained. A provenance is considered conserved *ex situ* if it is represented by at least 10 open-pollinated families with at least five trees per family at two different locations. The minimum number of different genotypes included in this scheme is 100 trees (20 families x 5 trees per family) with sample size based on molecular marker assessments of levels of genetic diversity and effective population size. The number of different genotypes protected is increased when dealing with critically endangered species like *Pinus maximartinezii*, which is native to only one location in Mexico, or for species with very low genetic diversity. If "genetically pure" population samples are needed in *ex situ* environments, crosses among families of the same provenance can be made in the future. As the trees approach biological maturity in their *ex situ* environment, they can be grafted onto rootstock and re-propagated. This low-cost conservation strategy has approximately a 40 to 50 year time frame.

One of the failures of *ex situ* conservation is that the genetic material given to the receptor country is seldom returned in some form to the donor country. Camcore has initiated a program to re-introduce seeds collected from its *ex situ* genetic trials from countries like Brazil, Colombia, and South Africa to Central America and Mexico. These re-introductions are now on a pilot scale as host countries determine if they can effectively manage local deforestation challenges to ensure the survival of the tree germplasm.

Ex situ tree conservation works best when the receptor country/organization has an environmental/economic reason for protecting germplasm. In the age of biotechnological advances, we have a tendency to forget that simple practices such as genetic thinning of trials and grafting are effective tools to maintain the health and genetic structure of tree populations. A cooperative approach to *ex situ* conservation reduces overall costs, but for it to be effective, it must be well coordinated. Every field trial and conservation planting in the Camcore program (2000 hectares) is identified with a test code that identifies location and genetic composition. As the trees in the Camcore *ex situ* conservation banks reach biological maturity over the next several decades, new (bio)-technologies will provide additional cost effective means for conserving tree populations.

Keywords: breeding, gene pools, alleles, productivity, disease resistance

Breeding Without Breeding: Approach, Example, and Proof of Concept

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Abstract

We propose an innovative framework called "Breeding Without Breeding" (BWB) to develop structured pedigreed material for breeding and testing. This method does not require any controlled crosses, relying on highly informative molecular markers (e.g., SSRs) and pedigree reconstruction methods. The high costs of breeding are replaced by lower-cost fingerprinting. Unlike classical methods used by forest tree breeders that rely on pre-determined mating designs to construct pedigreed materials for testing and selection, BWB allows the assemblage of full-sib (FS) and half-sib (HS) families from wind-pollinated offspring. BWB is tested and demonstrated using wind-pollinated seed collected from a subset of 16 maternal parents growing in a clonal Douglas-fir seed orchard. Despite a large seed orchard population (59 parents) and high pollen contamination (40%), 32 FS families were identified within the 16 maternal parents HS families using restrictive pedigree classification (95-99% confidence in paternity assignment). The efficiency of BWB was evaluated by varying the numbers of loci used for family classification (from 3 to 9). Families identified based on the 6 most informative SSR loci where very similar to those identified when all 9 loci were used. The identified FS and HS family structure was used in a nucleus population (NP) simulation to maximize the delivery of genetic gain from production populations. The proposed scheme resulted into substantial genetic gain increase ranging between 28-35% using forward selection within the NP, corresponding to a range of effective population sizes of 8 to13, as compared to the 8% of the original simulated seed orchard. Applications of BWB to breeding, conservation, and management of threatened and endangered species are discussed.

Keywords: Douglas-fir, genetic gain, microsatellites (SSRs), nucleus population, pedigree reconstruction, seed orchard, tree improvement.

Introduction

Tree breeders often select one or more traditional mating designs to create structured, pedigreed families to generate the material needed for testing and selection. In most cases a compromise over which mating design to use is necessary since no single design can meet all the goals and objectives set for each program (Namkoong, 1979). Characteristics of the ideal mating design are: 1) provides accurate estimates of parental genetic worth to select individuals to (a) create seed orchard or vegetative production populations (i.e., backward selection) and (b) select advanced generation parents; 2) produces sufficient crosses to select the best individuals from the best families for future breeding (i.e., forward selection); 3) estimates quantitative genetic parameters with sufficient accuracy and precision to determine the degree of genetic and environmental control and interaction influencing attributes under selection; 4) controls inbreeding through pedigree structure; and 5) achieves all these goals in a logistically and economically viable setting (Lambeth et al., 2001). These mating designs may take years to complete due to the large number of parents involved in a breeding program, delayed reproductive maturity, phenological differences, and gametic imbalances among selected parents (El-Kassaby, 2000). Additionally, crossing errors are frequently detected, mainly caused by pollen contamination and mislabelling (Adams et al., 1988; Devey et al., 2002). The diallel mating design (Griffing, 1956) is the only mating design that could meet the first four objectives, but the vast numbers of crosses required, even when partial diallels are used, presents logistical and economic difficulties. Using disconnected partial diallels reduces the number of crosses to a manageable level, especially when many parents are tested (Park and Fowler 1982; El-Kassaby and Park, 1993).

The polymix design is commonly used by tree breeders as a supplement to their primary mating design to evaluate the additive genetic worth of selected parents (Fowler, 1987). This complementary approach provides information no single mating design can, and is used by the major USA southern pine tree improvement cooperatives (McKeand and Bridgewater, 1992; White et al., 1993). The simplicity of polymix mating is appealing; however, the inability to infer the paternal contribution has been a major drawback (Wiselogel and van Buijtenen, 1988; El-Kassaby and Ritland, 1992). Lambeth et al. (2001) used 7 cpDNA and 3 SSR markers to successfully solve the paternity drawback, thus transforming the liabilities of the polymix mating (less genetic gain potential and limited potential for forward selection) into assets (simplicity, complete pedigree information, low cost).

We propose a novel approach to breeding where no controlled crosses are required. The framework rests on the data capabilities of highly informative DNA markers (e.g. SSRs) coupled with methods of pedigree reconstruction. In other words, this is an opportunity to conduct "**B**reeding **W**ithout **B**reeding" (BWB), where wind pollinated families are reconstructed and the costs associated with breeding are decreased and transferred to DNA fingerprinting (El-Kassaby, 2005). This approach is fundamentally different from traditional tree breeding methods. Its features could be extended to long-term breeding programs that may utilize sublines (i.e., independent replicates of a breeding population, where the primary advantage is to avoid inbreeding in production populations) (Lowe and van Buijtenen, 1986; White et al., 1993), as well as elite (nucleus) populations (higher effort concentrated on superior individuals in the nucleus relative to the main population) (Cotterill et al., 1989; Lstibůrek et al., 2004a, b). The proposed approach, if successful, could also be adapted to breeding methods for other organisms which utilize structured mating designs.

We describe and illustrate: 1) the framework for creating pedigree material without crossing, 2) a pedigree reconstruction example from a Douglas-fir (*Pseudotsuga menziesii*) seed orchard, 3) a method to optimize the laboratory work required, and 4) how the BWB concept can be extended to NP through simulations demonstrating a substantial increase in genetic gain due to this strategy.

Creation of Pedigree Material: Concept and Example

Second generation seed orchards (production populations) generally contain the best parents in the breeding population. When individual open-pollinated families are grown, seedlings within each maternal parent form a half-sib family (HS; sharing a mother). Furthermore, seedlings within each HS family form several FS families, with various sizes, sired by different paternal parents from either within the seed orchard or outside contamination sources. The genetic relationships among these families can be used to create a mating scheme across the mating landscape of the seed orchard using a matrix of relatedness. Individuals within each HS family can be grouped into FS families using: a) highly informative molecular genetic markers such as microsatellites (SSRs) and b) pedigree reconstruction methods (see Blouin, 2003 and Jones and Ardren, 2003, for reviews). Thus, a pattern of mating will emerge without conducting any crosses.

The BWB approach is illustrated using SSR data from a Douglas-fir seed orchard study (Slavov et al., 2005a). Ideally, seed orchards with a logistically manageable number of parents (≈30) and reduced pollen contamination levels are expected to increase the efficiency of the proposed approach; however, testing a system with imperfections provides a real assessment of the efficacy of BWB. The seed orchard population consisted of 59 clones (1-19 ramets/clone) with outcrossing rates of 98-99% and pollen contamination levels of 31-41% (Slavov et al., 2005a). All parents and offspring from 16 selected clones were fingerprinted using 9 SSR markers using a variable family sample size (mean: 36, range: 11-72) (Slavov et al., 2004, 2005a). Two paternity assignment methods were used to reconstruct FS families within each of the 16 HS families. First, we applied the likelihood-based assignment method of Marshall et al. (1998) using the CERVUS computer program (version 2.0). This method has certain limitations, particularly when pollen contamination is high (Oddou-Muratorio et al., 2003), but is expected to minimize the proportion of ambiguous paternities (i.e., cases in which paternity cannot be assigned because there are two or more compatible fathers). Paternities were assigned with 95 and 99% confidence, assuming that: 1) genotyping error was 1%, and 2) all potential fathers had been sampled. Second, we used the PFL computer program (Slavov et al., 2005b) to assign paternity by genotypic exclusion. We allowed up to two mismatching loci between the genotypes of offspring and potential fathers to avoid false exclusions caused by null alleles and genotyping errors (Slavov et al., 2005b). This approach is considered less efficient for assigning paternity because it cannot resolve ambiguous paternities. Our computer simulations, however, suggested that paternity assignment by exclusion may be very accurate when highly variable markers are used, even under high rates of pollen contamination and genotyping error (Slavov et al, unpublished).

The pedigree reconstruction identified individuals sired by: 1) seed orchard parents, 2) parents outside the seed orchard (pollen contamination), and 3) the subset of the seed orchard parents from which we sampled seed. These latter 16 parental clones were assumed to represent the NP for this study (Table 1, Figure 1). The 16-parent mating landscape will produce up to 256 FS families based on a full diallel design (n^2, where n = the number of parents). After eliminating selfs and combining individuals from reciprocal crosses to form an effective partial diallel, the number of possible crosses is reduced to 120 [n(n-1)/2] (Figure 1), increasing the size of the classified FS and HS families, thus improving the efficiency of pedigree reconstruction and substantially reducing the DNA fingerprinting efforts required to form FS families with "reasonable" sizes. A total of 48 FS families were produced (Figure 1).

Table 1: Seed orchard (SO) and nucleus population (NP) full- and half-sib classification success rate (excluding selfs) determined by pedigree reconstruction for Douglas-fir open-pollinated offspring using SSR markers and the correlation coefficient between NP success rate and the HS family size.

# of loci	**Removed locus PIC[1]**	**SO Success Rate**			**NP Success Rate (16-parent subset)**			**Correlation (NP & Family Size)**		
		CERVUS[2]		**PFL[3]**	**CERVUS**		**PFL**	**CERVUS**		**PFL**
		95%	**99%**	**95%**	**95%**	**99%**	**95%**	**95%**	**99%**	**95%**
9	--	58.3	42.7	57.6	24.7	19.8	26.6	0.79	0.82	0.80
8	0.799	56.4	38.4	62.0	25.0	19.4	27.4	0.78	0.70	0.79
7	0.844	57.8	36.1	60.9	26.9	20.8	28.5	0.74	0.68	0.81
6	0.858	55.2	34.0	58.3	24.3	20.1	32.8	0.68	0.59	0.80
5	0.888	46.5	21.7	39.8	22.6	12.7	35.9	0.64	0.52	0.88
4	0.899	37.0	17.4	20.1	20.3	10.8	36.8	0.67	0.61	0.92
3	0.915	15.1	2.3	N/A[4]	10.1	2.3	N/A	0.59	0.64	N/A

[1] PIC: polymorphic information content (Botstein et al., 1980) used for the sequential removal of loci. For example, the locus with the lowest PIC (0.799) was removed from the full set of 9 loci to obtain the set of 8 loci that were studied, and this process was repeated to obtain each successively smaller set of loci analyzed.
[2] Results based on likelihood-based paternity assignment using 95 and 99% confidence (Marshall et al., 1998).
[3] Results of exclusion-based paternity assignment allowing up to two mismatching loci between the genotypes of offspring and candidate fathers (Slavov et al., 2005a, b).
[4] PFL requires that the number of loci exceeds the allowed number of mismatching loci by at least 2.

When all 9 SSR markers were used, likelihood- (95-99%) and exclusion-based paternity assignment classified the 576 HS offspring into FS families with success rates of 58.3-42.7-% and 57.6%, respectively, for the 59-parent seed orchard (SO) population (Table 1). For the 16-parent nucleus population (NP), these success rates were 19.8-24.7% and 26.6% (Table 1). These results are very encouraging, given the: 1) high pollen contamination (mean weighted by family size = 40%), 2) large seed orchard population size (59 parents), and 3) small HS family size used (mean: 36 individuals/HS family). The reconstructed incomplete partial diallel design was produced from parents out of reproductive phenological synchrony (Slavov et al., 2005a). Phenological differences among individuals within a seed orchard stratify the parents into temporally, reproductively isolated sub-populations, thereby reducing the number of possible matings (El-Kassaby et al., 1984; Slavov et al., 2005a). The number of offspring assigned to FS families in the NP was significantly correlated with HS family size (Pearson correlation coefficients = 0.79-0.82 and 0.80, $P < 0.01$, for the two assignment methods), confirming that the power of BWB increases with larger HS family size (Table 1).

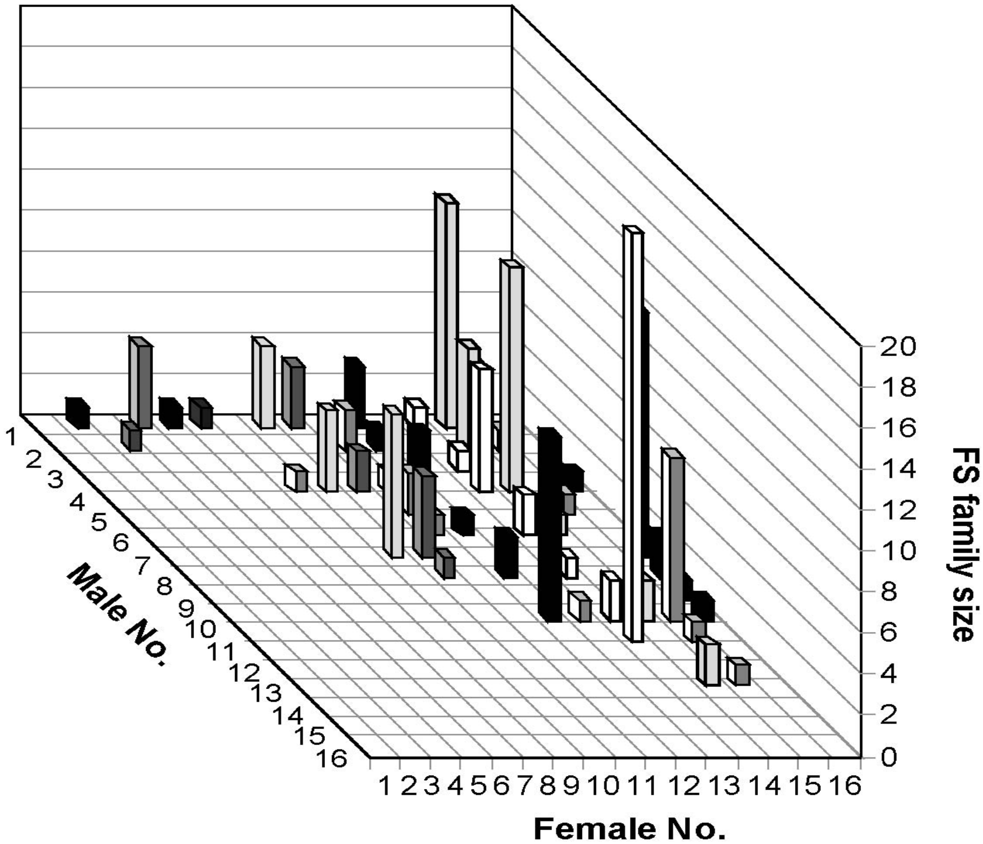

Figure 1. Likelihood- based progeny array classification (95% confidence) of the seed orchard's 16-parent subset (NP) into FS and HS families forming an incomplete partial diallel mating design.

Optimization of DNA Fingerprinting Efforts

We examined ways to optimize the laboratory time and expenses to produce the same results with fewer loci. Repeated analyses were conducted after sequentially removing the locus with the lowest polymorphic information content (PIC; Botstein et al., 1980) and reconstructing the pedigree (Table 1). The baseline 9-locus analysis was used as a benchmark for comparison within the NP (Table 1, Figure 2). Reducing the number of loci to 6 had a minimal effect on the family assignment success rate for either assignment method. The likelihood-based assignment method resulted in, significant Pearson coefficients of 0.985, 0.936, and 0.974 ($P < 0.01$) for 8, 7, and 6 loci, respectively, compared to the baseline, and all had similar numbers of classified individuals within the NBP (Table 1). Using 5 or fewer loci substantially reduced the success rate (Table 1), indicating that 6 SSR loci are optimal to produce results comparable to using 9 loci. Exclusion-based assignment produced similar results (Table 1). The only exception was that classification success in the NP increased with decreasing the number of markers used. With fewer loci, more offspring are falsely classified in FS families because of fortuitous matches to "incorrect" fathers. This is counterbalanced by the increasing number of ambiguous paternities. In the larger SO population (N = 59 parents), the effect of ambiguous paternities is stronger, whereas in the NP (N = 16 parents) classification success appears to increase with fewer loci because of the increasing rate of false paternity assignments. For both assignment methods, selecting appropriately polymorphic markers and an adequate number of loci is essential for maintaining a satisfactory and unbiased classification success rate.

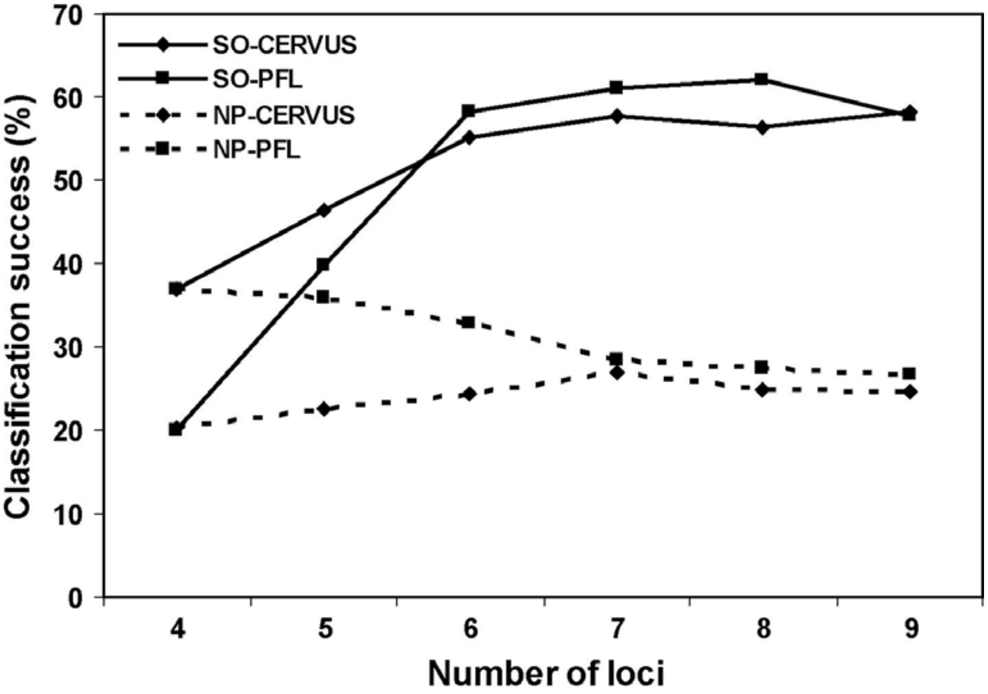

Figure 2. Success rates for likelihood- (95% confidence) and exclusion-based paternity assignment using different numbers of loci.

Effectiveness of BWB: Nucleus Population Application

The expected additive genetic merit of an individual can be calculated as the average of the parental additive values (Falconer and Mackay, 1996). The variation around this expectation in the second generation (no inbreeding) depends only on the value of additive variance of the unselected population (i.e., the larger the variance, the larger the dispersion of individual additive values around the mid-parent expectation). Selecting the top-ranking parents (based on predicted breeding values) therefore maximizes the distributional FS family means for future offspring population, creating opportunities to maximize the short-term delivery of high genetic gain through forward selection. The breeding scheme implemented here focuses on the top superior individuals in the seed orchard. We quantify the potential genetic gain through NP using a simulation approach and compare it to that of the original seed orchard using pedigree reconstruction. It is not necessary to rely on a pre-determined mating pattern to create the NP crosses; however, families created from BWB inside the seed orchard should produce an incomplete partial diallel design. This allows breeders to produce and deploy the highest possible gain in the shortest time possible with the resources available. This maximizes product differentiation between what is being delivered and what can be delivered, thus enforcing the benefits from breeding efforts and concentrates efforts towards managing the main breeding population.

Simulation Approach and Results

Simulations were conducted with a modified version of POPSIM™ (Mullin and Park, 1995). The strategy was initiated with the generation of a founder population of 100 individuals (Figure 3). The trait considered in this study was assumed to be influenced by a large number of independent loci, each contributing a small effect to its variation (infinitesimal model; Fisher, 1918). Using the parametric approach, individual additive genetic values were randomly sampled from a normal distribution $N(\mu_A, V_A)$, where $\mu = 0.0$ and $V_A = 200$. Environmental deviations were randomly sampled from a normal distribution $N(\mu_E, V_E)$, where $\mu_E = 100$ and $V_E = 300$, giving rise to a narrow-sense heritability $h^2 = 0.4$. Individual phenotypic value was the sum of the independent additive effect and environmental deviation.

All individuals in the founder population ($n = 100$, $N_S = 100$) were unrelated and non-inbred (N_S: status number – the definition is provided below). Founders were sorted by their respective phenotypic values and the top 40 were selected to form a seed orchard ($n = 40$, $N_S = 40$). To maximize genetic merit of the future seed orchard, the 15 top performing individuals from the orchard (also based on their phenotypic performance) were selected to form the nucleus population (Figure 3). Half-diallel mating was performed among these NP parents and 100 offspring genotypes were generated within each parental combination (105 full-sib crosses). The individual additive value of each offspring was randomly sampled from $N(a_{FS}, 0.5V_A)$, where a_{FS} is the mid-parent additive value. Forward selection of the top 40 individuals from the progeny test was then performed to establish a new orchard (Figure 3) using group-merit selection (Lindgren and Mullin, 1997) considering both breeding value and relatedness. The algorithm iteratively maximized the population merit: $B_\omega = g - c\Theta_\omega$, where B_ω is the group merit of a selected set ω, g is the average additive genetic value of the set, Θ_ω is the group coancestry (Cockerham, 1967) of the set, and c is a weighting constant. The weighting constant was varied in each simulation between zero, the extreme when only genetic gain was the selection goal, and infinity (approximated by 1.0×10^7), when the group coancestry of the selected set was minimized. One hundred simulation interations were run to predict genetic gain of the new seed orchard. Status number (N_S) is used as a measure of the effective population size: it is the census size of an equivalent population composed of unrelated, non-inbred individuals, where the probability of randomly drawing two genes that are identical by descent is the same as for the whole population (Lindgren et al., 1996). Status number was calculated from the group coancestry ($N_S = 1/2\Theta$). Using N_S enabled comparisons of how much genetic gain is sacrificed per unit of effective population size of the selected orchard due to more restrictive selection. To assess the variability among simulation scenarios, 95% confidence intervals were calculated and presented along with the average additive effect (genetic gain) and status number (Figure 4).

Simulation results are presented in Figure 4, where 0.0% genetic gain is a reference line that corresponds to the mean additive value of the 100 founder genotypes ($N_S = 100$). The dashed horizontal line at genetic gain of 8.6% corresponds to the gain by selecting the 40 best individuals (i.e., the original seed orchard ($N = 100$)) and the solid lines above and below represent the 95% confidence intervals (Figure 4). The value of 8.6% can be calculated deterministically: selection intensity = 0.97, $V_P = 500$, gain app. 8.6%.

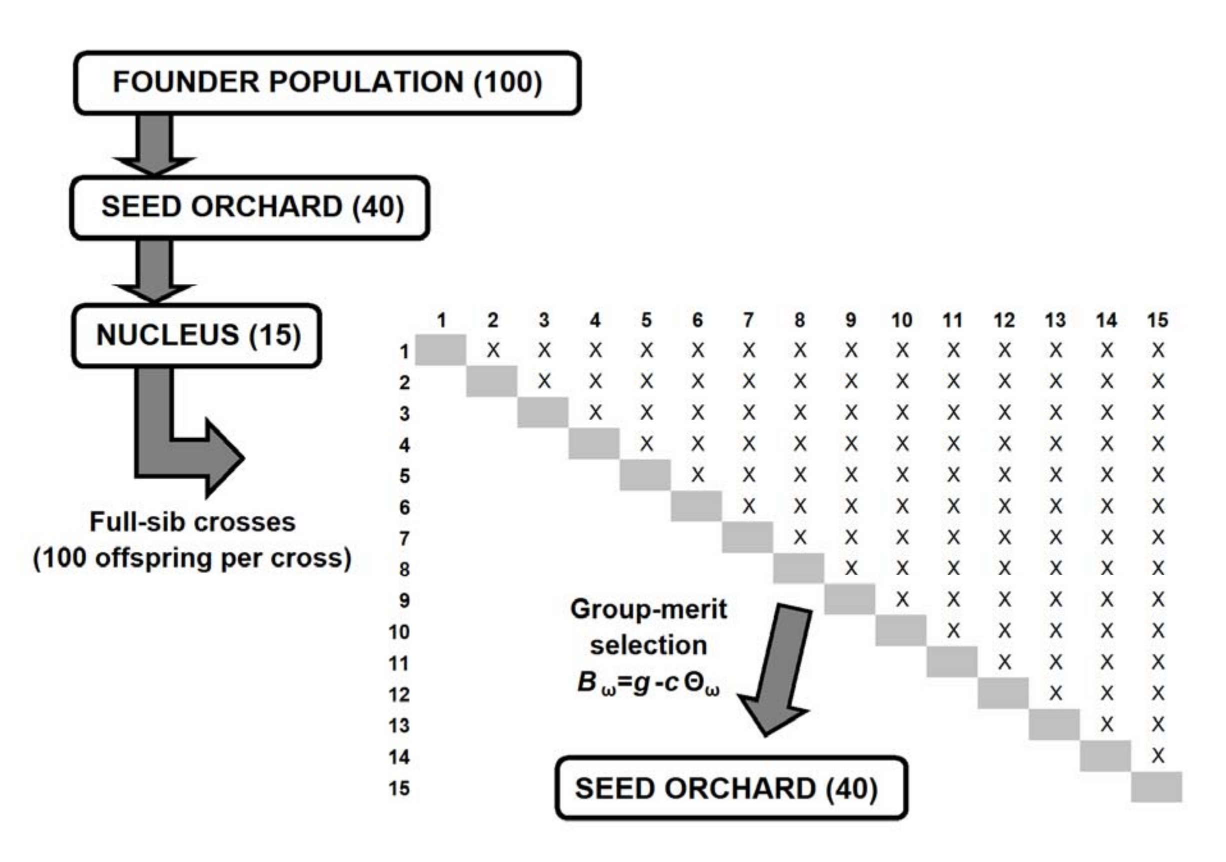

Figure 3. Schematic description of the breeding strategy implemented in the simulation. The founder population is equivalent to a phenotypically selected breeding population in which the top 40 parents form the original seed orchard population and the partial diallel represents the FS and HS produced through pedigree reconstruction and the selected best individuals with these families form the parents for the new production population (seed orchard).

The new seed orchard produced from the NP had higher gain (28.2-35.0%; Figure 4) than the original seed orchard. The level of gain was affected by the importance assigned to gene diversity measured by effective population size (N_S = 8.1-12.5). Under any deployment strategy, the delivered gain far exceeded that of the original seed orchard, and the reduction in gain was related to the corresponding effective population size. An effective population size of 12.5 represents the maximum balanced parental contribution, while 8.1 correspond to selection criterion where the additive genetic merit of the selected orchard is the only component. Even under this scenario, the selections are still dispersed among families due to the high gain captured from the large within-family variance.

It should be stated that the magnitude of genetic gain illustrated from the NP simulation above is based on complete partial diallel (i.e., "balanced" mating design); however, similar gain can be captured if the partial half diallel is incomplete. When positive assortative mating is conducted among high breeding value parents, differences among crosses are very small compared to that within crosses, thus the expect genetic gain is expected to remain high from incomplete partial diallel. Genetic gain comparisons among and within families produced from positive assortative mating are presented in Lstibůrek et al. (2006). This situation highlights the importance of HS families sample sizes used for the pedigree reconstruction and the accuracy of pedigree reconstruction itself.

Utility of BWB

In addition to the role of BWB in tree improvement programs illustrated above, both full and partial pedigree reconstruction can play significant roles in conservation genetics. The advantage of inferring relatedness among individuals in a natural setting will produce a matrix of genetic relatedness among individuals that could be used to determine the genetic control of adaptive and quantitative attributes without the need to grow progeny, essential for the development of sound gene conservation strategies. Field estimates of adaptive and quantitative attributes are very important since they avoid some of the confounding issues related to experimental environments such as limited competition, lack of stress, and maladaptation of off-site sources (Ritland, 2000). BWB could also be used to manage genetic resources of threatened and endangered species where preserving the genetic diversity is the main goal. Population management incorporating pedigree-based genetic management to identify the relationships among captive individuals would help avoiding or reducing the buildup of inbreeding while conducting breeding programs (Russello and Amato, 2004).

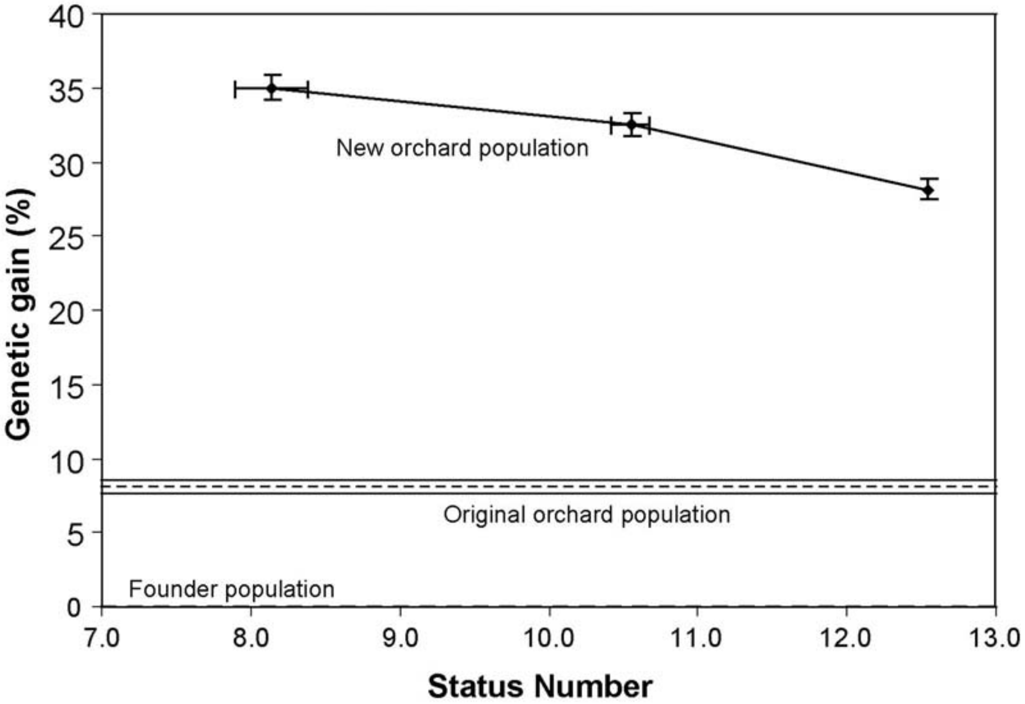

Figure 4. Genetic gain estimates for the founder population (0.0% gain), original seed orchard (8.6% gain ± 95% CI), and for the newly established seed orchard under various effective population sizes (CIs based on 100 simulation iterations).

The added value of the pedigree reconstruction in the proposed BWB strategy is to confirm the actual parentage of an individual, where both parents belong to a given seed orchard (or a subset of the orchard). It is therefore possible to eliminate individuals with unknown or undesirable male parent and exclude them from further testing. Knowledge of the full relationship matrix can also be utilized in selecting the new seed orchard (e.g. Figure 3). Given that open-pollinated families are tested without the parental confirmation, much larger testing resources would be required (no pre-selection of offspring individuals belonging to the nucleus diallel mating scheme). On the other hand, selection based purely on individual performance (relatedness not included in the selection criterion) does not reduce dramatically the effective population size of the future seed orchard as indicated in Figure 4.

Conclusion

The conceptual framework for breeding without breeding (BWB) is presented and illustrated using SSR markers on a wind-pollinated family array from 16 parents growing in a Douglas-fir seed orchard. The importance of proper marker selection and how to optimize laboratory efforts are illustrated. BWB is a good complement to conventional tree improvement programs that would expedite the delivery of high genetic gain. Finally, the utility of BWB in gene conservation and the management of threatened and endangered species is highlighted.

Acknowledgments

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Economic Gains from Tree Improvement: An example from Turkish Red Pine (*Pinus brutia* Ten.) Plantations in Turkey

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Abstract

One of the important objectives of plantation forestry is to maximize gains from a unit planted area. Silvicultural treatments, such as site preparation, planting techniques, stand management, and planting genetically improvement material are some of the issues need to be addressed to increase the gain. The aim of this paper is to estimate the economical gain from planting genetically improvement material in plantations in Turkey. Software for economical analyses of plantation (PLANTEK) was developed and applied on different plantation sites. All the expenses and incomes during the rotation period were considered and three investment criteria, namely, the net present value, input/output ratio and internal rate of return were used for economical evaluation. Comparisons were also made between plantation areas where genetically improved and unimproved seed sources were used. The results showed significant impact of planting genetically improved stock on economic gains from Turkish red pine plantations.

Keywords: fast growing species, industrial plantation, economic analyses, cost benefit analyses

Conservation of forest genetic resources and climate change: do current strategies provide the right answers?

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Extended Abstract

The forestry community is now well aware of the necessity to conserve the genetic variability of natural forests to maintain their adaptability in the long run. Since the Earth Summit of 1992, and as a result of the MCFPE process, European countries have designed a collaborative network to protect their forest genetic resources: Euforgen. In parallel, countries have set up various national mechanisms to protect their forests. In France for example, *in situ* conservation networks have been implemented for priority forest tree species threatened for different reasons, based on current ecological and genetic knowledge (Teissier du Cros et al. 2001). There, conservation units form a network which sample the biodiversity of the target species and contain autochthonous populations that are managed under simple “genetically-sound” rules. As knowledge increases, new populations can be added to species networks and new target species can be addressed. With such a system, natural populations evolving under current ecological constraints can be found for every ecotype the species has.

Can such networks conserve an evolutionary potential sufficient to face climate change ?

Tree species can use three types of processes to react to environmental changes: they can acclimatize, adapt and/or migrate. Experimental work has shown that because forest tree populations retain a high level of variability for most adaptive traits, their plasticity is often great and makes it possible for them to acclimatize (e.g. Matyas & Yeatman 1992). Although genetic variability is often strongly geographically structured, and local populations adapted to their local environment, high within population diversity also makes it possible for adaptation to occur even over a single generation (e.g. Skroppa & Kohmann 1997). Gene flow by pollen is also an efficient way to disseminate adapted genes over large distances in tree species. Studies combining paleoecological and genetic data have also shown that trees can move rapidly over large distances per generation through movement of seeds (e.g. Magri et al. 2006).

However, the changes expected are probably much larger and much quicker than those which affected natural populations during the Quaternary climatic cycles. Scenarios proposed by the Intergovernmental panel on climate change (IPCC) indicate that temperatures are predicted to increase by 2–4°C in Europe over the next 50 years. They also indicate that precipitations are predicted to change their regional and seasonal patterns dramatically. Most of all, they show that extreme climatic events (heat waves for example) will be on the rise with mostly rather unpredictable patterns. Current genetic data based on multi-species range-wide studies have

shown that climate changes can affect within species diversity, mostly by producing genetic drift (e.g. Fady 2005 for Mediterranean conifers).

Adaptation processes will certainly occur within conservation units. However, due to the rapidity and local uncertainty of predicted climate changes, the question of whether they will serve their purpose for their target species within an assigned territory is debatable.

- First of all, not all forest tree species are found within these networks. Which new species should be added needs to be assessed based on climate change considerations in addition to the current ecological and economical considerations used. For example, species affected in the past by climate change and demonstrating a reduced genetic diversity following demographic bottlenecks might be privileged targets.

- Second, for those species included in conservation networks, the need to include new populations (whether disjunct or marginal for example) also needs to be assessed based on climate change considerations. In the Mediterranean, for example, populations growing under xerothermic conditions demonstrate reduced genetic diversity although they may contain those genes needed for future adaptation to drought conditions.

- Third, trapped within fragmented landscapes, some conservation units may completely disappear. In such cases, local genotypes are not enough and gene flow from other, possibly from more drought resistant populations, whether from the same species or from related species, needs to be considered. Multi-species management and promotion of hybridization may be an answer, although it is certainly not a currently recommended practice.

- Fourth, management of target species, for example the density at which adult trees are left at the time of stand regeneration, influences the genetic structure of seedlings, i.e. the level of consanguinity within an area. Multiple density thinning strategies might be needed to promote maximum diversity within conservation stands.

- Fifth, once a network is installed, the way genetic diversity evolves through time needs to be monitored. Devising the most appropriate (both scientifically and technically speaking) within species biodiversity indicators remains a challenge.

- Finally, considering the speed at which climate is foreseen to change, movement of populations outside of their current range may well need to be considered. Local populations will not always be best ! In this respect, ex situ conservation should be considered as an integral part of in situ conservation of autochthonous species for an efficient adaptive conservation.

In conclusion, Man may have to act boldly to prevent local disasters and increase the spread of adapted genes in soon to be threatened populations of widespread forest trees species, possibly by devising management strategies that will go against currently operational regulatory mechanisms.

Figure: The Lure mountain in southern France, a conservation unit for the southernmost ecotypes of *Abies alba* in the Alps.

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Return of the Giants – Lessons from a High Input Restoration Program

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Abstract

In the late 19th century, western white pine *(Pinus monticola)* dominated the moist, mid-elevation, mixed species forests of the Inland Northwestern United States and Interior British Columbia. By the late 1960s, poor management practices (high-grading, over-harvesting and fire suppression), mountain pine beetle infestations, and infection by the exotic rust fungus that causes white pine blister (*Cronartium ribicola)* had, in combination, decimated these once-majestic forest stands, leaving less than 10 percent of the historic 5 million acres of white pine cover type in Inland Northwestern forests.

Early research efforts to find a genetic solution to the rust problem predicted a 50% gain in seedling survival in the F_2 generation compared to seedlings from general forest collections. But results of a 1966 progeny test conducted in a nursery setting exceeded those predictions, with 66% of the F_2 seedlings remaining canker-free at 2½ years after a single inoculation in the nursery.

In recent studies, rust infection in operational field plantings of F_2 stock averaged 36%, close to the levels reported in the 1966 nursery test. But the high variability in infection levels between sites suggests that long-term rust resistance levels may be somewhat lower than reported for the young seedlings in the 1966 test. Nonetheless, the large differences in infection levels and mortality between the selectively bred F_2 stock and the controls, and the relatively consistent performance of trees with the different resistance traits, clearly demonstrate that substantial gains were achieved in the early breeding program.

Although this program currently uses a high-input, long-term strategy for development and testing of genetic materials, the early work was conducted on a modest scale. Critically important was the foresight of the early scientists in conserving and out-planting their materials in field sites, thus providing opportunities for long-term assessments of performance, for further breeding for resistance and for evaluating the durability and physiological basis of specific resistance traits. Furthermore, these trees may be good candidates for verifying the validity of genetic markers for resistance, should they become available.

Key Words: blister rust, western white pine, long-term evaluation, durability of resistance

Low Input Improvement of Christmas Trees

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Abstract

The Christmas Tree Genetics Program at N.C. State University started July 1, 1996 with the charge to improve Christmas tree species important to the state. Emphasis of the program is on Fraser fir (*Abies fraseri* [Pursh] Poir.) which is grown at elevations above 1,000 m in the western N.C. Mountains; however, other minor species are grown as Christmas trees in the N.C. piedmont and coastal plain regions as well as throughout the Southeast U.S. Due to their lower economic value and volumes produced, intense genetic improvement programs are not economically justified for these species. Low input programs developed for two such species, Virginia pine (*Pinus virginiana* Mill.) and eastern redcedar (*Juniperus virginiana* L.) will be discussed. In both cases, clonal propagation of select trees through rooted cuttings is being employed to increase both the uniformity and genetic gain of the planting stock.

Keywords: *Pinus virginiana*, *Juniperus virginiana*, vegetative propagation, clonal propagation

Genetic and Physiological Traits of Diploid Progenitors and Newly Synthesized Polyploid Trees

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Abstract

In this study, we examined unmodified (diploid) and modified (putative polyploid) lines of tree species *Agathis robusta* (Araucariaceae), *Elaeocarpus grandis* (Elaeocarpaceae), and *Paulownia tomentosa* (Scrophulariaceae) that were generated with a new laboratory procedure that allows mass production of putative polyploidy clones. *Agathis robusta* and *E. grandis* are native to Australia while *P. tomentosa* is a Chinese tree species. These species have potential to be used as timber species or are already being used in Australian forestry. The unmodified and modified lines were subjected to three sets of investigations: (1) verification of polyploidy using a combination of flow cytometry and chromosome counts, (2) verification of genome stability using AFLP, a total genomic marker, and (3) characterisation of leaf anatomy, morphology, physiology, and growth using microscopy, in vitro analyses and in vivo measurements including maximal CO_2 fixation and electron transport rates.

Plants of unmodified and modified clone lines, approximately 10-15 cm in height were grown for three to six months in a naturally lit glasshouse and were examined for genetic, anatomical, physiological, and growth traits. Not all species underwent all investigations because insufficient plant material was available for physiological studies of *E. grandis* and further methods development is needed for successful flow cytometry of *A. robusta*. The aim of the study was to confirm that the modified clones were indeed polyploid, and if confirmed, to determine which new properties is the result of polyploidy in the studied tree species when grown under controlled conditions.

Results showed that the nuclear DNA content in cells of *E. grandis* and *P. tomentosa* clones (the two species successfully tested) were up to 35% higher than in parent plants, consistent with individuals experiencing genome duplication. AFLP results showed that all studied clone lines of *A. robusta, E. grandis*, and *P. tomentosa* had high genomic stability demonstrating that mass clonal production programs should produce phenotypically stable clone lines. However, most of the clone lines tested here had slightly divergent genotypes possibly resulting from sight genome rearrangements and indicating that all clones lines have resulted from an independent polyploidisation process. These results highlight that the new polyploidisation technique that was used here may create novel genetic variation, which could be very useful for breeding programs.

Selected modified clone lines differed in leaf anatomy, morphology and physiology from the parent plants of *A. robusta* and *P. tomentosa*. Selected clone lines had statistically significantly larger and thicker leaves with 18 to 27 % greater stomatal aperture length than their parental lines, respectively (Figure 1). Leaf morphology and growth differed in selected clones from parent plants. Selected clones had higher leaf elongation rate, greater leaf area of single leaves and greater total leaf area per plant. While not always statistically significantly different, there was a consistent trend for increased biochemical properties (higher total chlorophyll content, higher soluble protein, and greater leaf nitrogen-use efficiency) for clones of *A. robusta* and *P. tomentosa* compared with diploid parents. The different leaf biochemical properties of clones compared with parents were also reflected in leaf physiological properties. Selected clones had significantly higher rates of maximum CO_2 fixation, maximal rubisco carboxylation, and maximal electron transport than parent plants. In *A. robusta*, the putative polyploid clone had accumulated significantly more shoot biomass (main stem and branches) than the parent after six month of growth in the glasshouse.

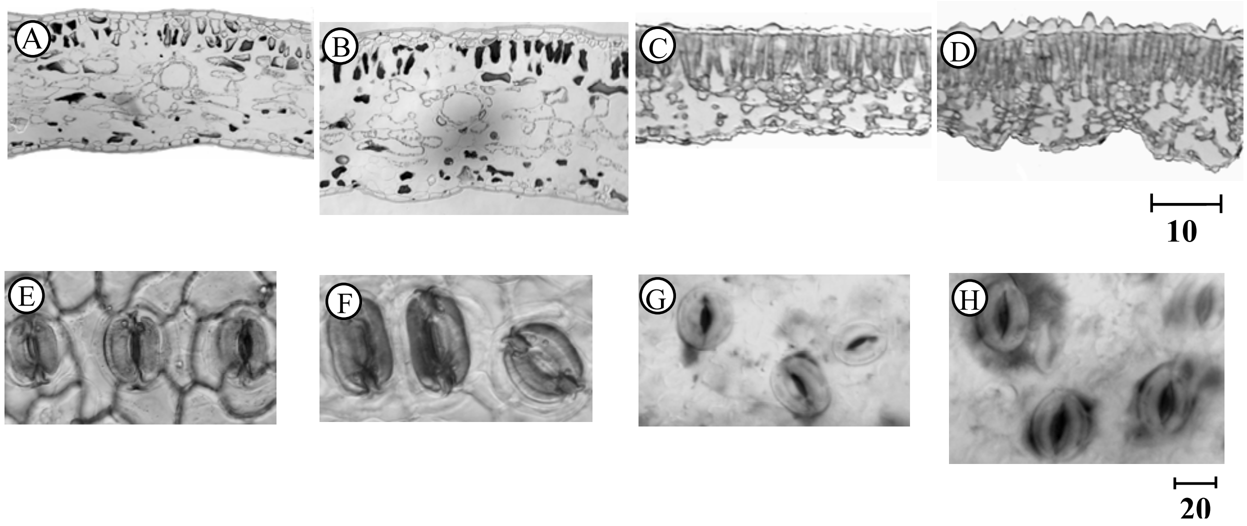

Figure 1. Photomicrographs of leaf cross sections and stomata on the abaxial epidermis of parents and clones of *A. robusta* and *P. tomentosa,* species after 3 and 6 months growth in the glasshouse, respectively. *Agathis*: A and E, parent; B and F, clone; *Paulownia*: C and G, parent; D and H, clone. Photos of cross sections and stomata were taken using a digital camera attached to a light microscope under 100 x and 200 x magnifications, respectively.

Taken together, the findings of consistent differences in nuclear DNA content, leaf properties, and, in the case of *A. robusta*, growth in diploid progenitors and newly generated lines of three tree species confirm that the new lines are polyploids. Further investigations are now required to determine the reasons for the observed increase but not doubling of nuclear DNA content in modified clones. Two possible reasons include that either a large amount of the doubled genome is lost soon after the polyploidisation process, or that ploidy reversion occurs during development in polyploids. Whatever the reason is, our findings strongly suggest that the modified clone lines have undergone a successful polyploidisation process that has resulted in increases in nuclear DNA content and that the modified clones exhibit traits similar to those found in previous studies of polyploid *versus* diploid taxa. Further research is now required to test the environmental resilience and growth of the polyploids in the field.

We conclude that the new procedure of genome duplication of trees may offer exciting opportunities to develop stable polyploid trees as timber crops with improved growth performance and environmental resilience.

Key words: genome stability, growth, leaf anatomy, morphology, physiology

The use of parental reconstruction in the breeding programme for *Eucalyptus nitens*

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Abstract

Increasing the efficiencies of an open-pollinated breeding strategy for *Eucalyptus nitens* in New Zealand was addressed using microsatellite markers to reconstruct the parent identity of forward selections. Microsatellites (SSR) are ideal markers to reveal genetic relationships between individuals owing to their co-dominant inheritance. The successful application of these markers to identify the parentage of open-pollinated progenies has had major implications to the breeding strategy and seed orchard management of *E.nitens*.

The forward selection of individuals for the breeding population and future deployment was simulated using 10 open-pollinated offspring from each of 10 seed parents in a clonal seed orchard. A set of fifteen SSR markers from a total of 41 initially tested was used to reconstruct the parentage of the progeny. Ninety percent of the progeny samples matched consistently to a single mother and father and among these 13 were selfs. Eight percent had a maternal match only; this would indicate that either there was contamination by pollen from outside the orchard or there was a failure by the marker set to identify the orchard parent. One percent had no maternal match and one percent could not segregate between two fathers.

The estimate of the coefficient of inbreeding and coefficient of coancestry were derived for the seed orchard and the forward selections.

Keywords: microsatellite markers, genetic gain, progeny testing, pollination

Rehabilitation the juniper forest ecosystems in terms of their present silvicultural situation in Turkey

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ABSTRACT

Turkey has a total area of about 78 million ha, of which around 25% is classified as forest land. Juniper forests cover 6% of this total area of forests and consists of seven juniper species. Some of the species are very important due to their valuable timber. Furthermore, some parts of the tree species play important roles in human being health. In addition, the species are used to prevent soil erosion and design parks and gardens of the country, too.

However, as a result of centuries long degradation (fires, overgrazing and encroachment for farming, excessive cutting) 93% of the juniper forests are currently under degraded and unproductive conditions and far from providing expected economical, ecological, social and collective-cultural functions and services. These degraded and unproductive juniper forest lands are subject to erosion of different intensities.

This paper deals with the description, natural distribution, silvicultural characteristics, environmental requirements, yield, establishment by natural and artificial regeneration, pests and diseases, wood properties and uses of products of Crimean juniper (*Juniperus excelsa* Bieb.) and Phoenician juniper (*Juniperus phoenicea* L.) species in Turkey. Regarding juniper forests, as a genetic heritage of the human being, recommendations related to rehabilitation and protection measures to be taken for the existing of juniper forest ecosystems of Turkey in short and long-terms are evaluated.

Keywords: Degradation, genetic heritage, protection, evaluation.

1. INTRODUCTION

Juniper forests cover an area of 925.822 ha, which is 11% of the total area of coniferous forests in Turkey (Gezer ve Yucedag, 2006). According to the latest forest surveys, 70,515 ha of the total forest area is high productive forest and the rest (855,307 ha) is under unproductive conditions (Konukcu, 2001).

Genus of Juniper is named as “Ardıc” in Turkish corresponding to the word of “Rear Guard” which is because of its coming after diminishing of any forest ecosystem. As the species are drought tolerant trees that withstand aridity and poor soils better than most timber species grown in Turkey. The species are most widely distributed trees of the Mediterranean basin, particularly in the western and southern part of the country.

The genus is represented by sabin and oxycedrus sections as to their female flowers, especially cone scales and leaf forms characteristics.

The main species of oxycedrus section are *Juniperus oxycedrus* L. and *J. communis* L. together with the subspecies *J. oxycedrus* L. subsp. *oxycedrus,* subsp. *macrocarpa* (Sibth. & Sm.), *J. communis* L. subsp. *hemisphaerica* (Presl) Nyman, subsp. *nana* Syme.

Sabin section consists of *Juniperus excelsa* Bieb., *J. phoenica* L., *J. foetidissima* Willd. and *J. sabina* L. species in Turkey (Yaltırık, 1993).

In this paper, focus is especially upon Crimean juniper (*Juniperus excelsa* Bieb.) and Phoenician juniper (*Juniperus phoenicea* L.) which have their greatest distribution in southern Turkey where they have become the most valuable commercial tree species.

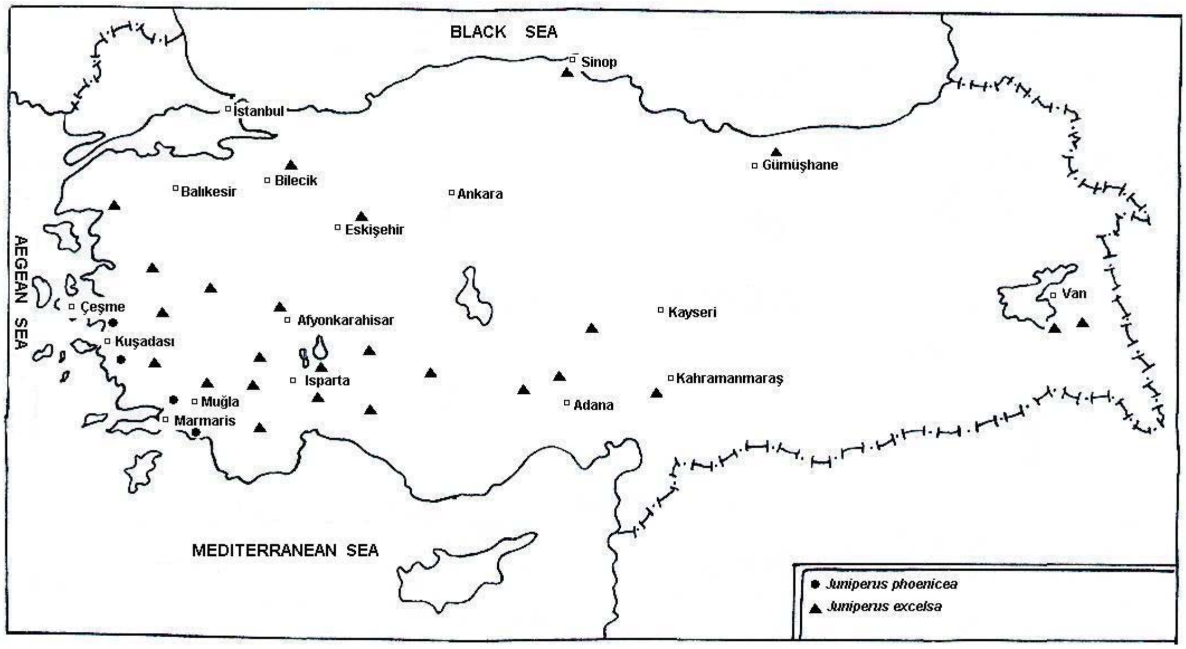

Figure 1. Natural Distribution of Crimean and Phoenician Juniper Species in Turkey (Updated from Davis, 1965 and Elicin, 1977)

Crimean Juniper: The species has a wide distribution in Turkey. It grows mainly in extensive stands in southern and western Anatolian. It is also found in the Black Sea, Middle and East Anatolian Regions. The species reach the greatest commercial importance on Taurus Mountain of the southern Anatolian region (Figure 1). The species occupies rocky and stony slopes of the mountains (Elicin, 1977; Ansin and Ozkan, 1993).

Crimean juniper is a light demanding tree. It is resistant to fire (Aykın, 1978). The species in terms of these characteristics is similar to Anatolian black pine (*Pinus nigra* Arnold. subsp. *pallasiana* (Lamb.) Holmboe). It is fast growing tree between the ages of 50 to 70. Mature trees are generally 25 m tall, but may reach as high as 30 m, with trunk diameter up to 80 cm. The species grows between the altitudes of 500 to 2000 m in Turkey. The taxon constitute pure and mixed stands with Taurus Fir (*Abies cilicica* Carr.), Anatolian black pine, Lebanon cedar (*Cedrus libani* A. Rich.), Brutian pine (*Pinus brutia* Ten.) and some broadleaved tree species

(*Quercus* spp. etc.). The Crimean juniper occasionally occurs to grow in maquis belt in which some deciduous and evergreen trees, shrubs such as *Q. infectoria* subsp. *boissieri*., *Cercis siliquastrum* L., *Fraxinus ornus* subsp. *cilicica*, *Ceratonia siliqua* L, *Fontanesia phillyreoides* L., *Q. coccifera* L., *Q. colliprininos* L., *Pistecia terebintus* L., *Olea europea* var. *oleaster* L., *Erica verticillate* L., *Ctinus coggygria* L., *Spartium junceum* L., *Vitex agnuscastus* L. associate with the tree species.

Phoenician Juniper: The species is a restricted to the southern and western Mediterranean Region. It grows from sea level to the elevation of 1200 m in its natural (e.g. surrounding of Mugla, Marmaris, Kusadası-Samsundagı and Izmir-Cesme localities) distribution (Figure 1). It grows exceptionally fast with strait trunk and crown and it may reach a height of 15 m (Elicin, 1977).

The species is frequently a dominant forest tree in the Mediterranean forest region. It forms very small pure groups among the maquis belt which covers large areas below 400 meters along the coast and in depression places penetrating deep into the inland. The leading most common species associated with Phoenician juniper are *Q. infectoria* subsp. *boissieri*., *Styrax officinelis* L, *Pistacia lentiscus* L, *Pyrus salicifolius* L, *Arbutus andrache* L, *Cercis siliquastrum* L, *Rhus coriaria* L, *Juniperus oxycedrus* L, *Phillyrea lotifolius* L, *Cistus creticus* L. and *C. salvifolius* L. Above the elevation of 800-900 meters junipers are replaced by *Pinus nigra* Arn. var. *pallasiana* (Lamb.) Holmboe and *Cedrus libani* A. Rich. in its natural distribution area.

The genus has a great importance due to its timber used in a very wide range of applications from fencing posts through railway sleepers and saw logs for box making, general construction and joinery industries, and the wood of some Juniper species are also suitable for naval industry and stationery. Furthermore, some parts of the species play important roles in health; they are used in chemical and medical industry due to their organic and inorganic components. In addition, the species are used to prevent soil erosion and as ornamental trees in parks and gardens of the country.

However, as a result of centuries long degradation (fires, overgrazing and encroachment for farming, excessive cutting) 93% of the juniper forests are currently under degraded and unproductive conditions and far from providing the economical, ecological, social and collective-cultural functions and services expected from them. It is vital/important to afforest such kind of degraded forest lands by using seeds of suitable origin which have adaptability to be climate conditions, soil properties and physiological characteristics of those lands. The juniper species, undoubtly are the important species to be taken into consideration.

In this paper, environmental requirements, seeding habits, management and regeneration systems consisting of seed production and nursery works, of the species have been reviewed. In addition, under headline of “Conclusion” measures to be taken in short and long terms, in order to solve chronicle problems of the species connected with these activities have been discussed.

2. MATERIALS AND METHODS

2.1. Environmental Requirements

Climatic: The Crimean juniper is the unique species that covers and forms pure stands on extensive areas of interior and exterior slopes of Taurus Mountain. Crimean juniper extends from subhumid zone of Mediterranean region to parts of the adjacent semiarid steppe zone. In other words, the species is adapted to sites normally unsuited for other crops because of infertility, aridity or topography. It is a drought tolerant tree that withstands more aridity and poor soils than most timber species growing in Mediterranean climate.

The species is able to grow in conditions ranging from the low altitude maritime climate to high altitude steppe climate. In other words, the species can be found from low altitude costal region up to high altitude steppe.

According to the climatical data obtained from the nearest meteorological station to the most important natural distribution of juniper, which is classified as juniper belt located within boundary of Sutculer forest districts, are given below.

According to the mean monthly climate data obtained from registered values of Egirdir Meteorological station (950 m a.s.l.), which is about 50 kms away from Sutculer district, are used to determine the climate values of the focus area. According to climate values of the distribution of Juniper (1450 m a.s.l.), the average annually precipitation is 924 mm and 26% of this precipitation is in spring, 11% is in summer, 17% is in autumn and 46% is in winter. The average annual temperature is 11 ºC. In juniper spreading area, the hottest month is July and August, during these months, the average temperature is 21,6 ºC. The maximum temperature is 32 ºC in July; the minimum temperature is -12,2 ºC in February.

The climate type is semi-humid and the vegetation cover is dry forest like park appearance (Erinc, 1965).

The growing-season period varies from 160 to 200 days and over; it is obvious that low temperature and snow fall are the main ecological factors limiting Juniper from spreading beyond the defined habitat; in its distribution area/natural range.

Soil: The soil conditions of natural range of Juniper in Turkey have not been fully studied yet. However, it is known that the species is hardy and highly adaptable to various types of soils. It can grow on shallow and stony soils. The dominant soil types are red Mediterranean and calcareous brown forest soils. It is indicated that the origin of soil does not considerably affect its growth. In southern Anatolian, Juniper forests grow on calcareous soils which have sufficient depth and moisture. In addition to this, the tree may grow in different types of soils originated from andesite, sandstone, schist, granite and, rarely, on serpentine. It grows in soils with a wide range in structure varying from sandy clay; sandy loam to silt loam, but it prefers sandy and loamy soils for it enables the best development. Juniper can grow at poor areas where other forest trees can not grow.

The species grows well on alkaline and physiologically shallow soil. In general, it prefers the sunny slopes but susceptible to snow damage and inconvenient conditions (bad drainage conditions) of drained soil in juvenile stage (Pamay, 1955).

Causes of Damages: Factors affecting the management of Juniper forest are various and extensive in Turkey. The ways of forest destruction range from encroachment for farming to entomological factors, and the role of fire and grazing in diminishing the forests are extensive.

Damage by sheep, goats and rabbits causes serious increment losses to young juniper trees when they get about the ground vegetation but are not high enough for their leading shoots to be out of reach. However, the tree can rapidly replace damage shoots, but this response varies with provenance and locality. The juniper, even if held in checked for several years by grazing, soon grow new shoots when grazing pressure is reduced by fencing out sheep, goat and rabbits. In addition, seedlings are eaten by small rodents such as mice and squirrels.

Juniper is normally resistant to both late and early air frost but damage to the foliage sometimes occurs and susceptibility is probably dependent upon the age and provenance of plants.

In severe winters the leaves of seedlings (one or two years old) sometimes turn brown, particularly on exposed nurseries, usually followed by decrease in growth in the following year.

On the other hand, there is also some illegal cutting in Juniper forests which creates difficulties in operating a managed forest.

Disease and pests are not a major problem for Juniper stands. There are a number of insects and fungi attacking Juniper; but the important ones do not exceed few, which are mentioned as in the following.

Insects damaging Juniper species are:

Megastigmus juniperi Nikol'skaya, live into the seeds and the larvae feed by endosperm and embryo of Crimean Juniper. However, the insect's population is not very serious in the natural distribution of the species.

The most serious fungal diseases are:

Fomes juniperus (von Schrenk) Sacc. and Sydow, has been determined in *Juniperus excelsa* and *J. foetidissima* species. This fungus causes brown rot.

Gymnosporangium fuscum DC., cause young sprout dead and blight in bud and fruits of Juniper species.

Gymnosporangium clavariaeforme (Jacquin) DC., causes damages and deformations only on branches and stems of *Juniperus oxycedrus* (Canakcıoğlu and Elicin, 1999).

On the other hand, damping-off is the most serious disease for one or 1.5 months old seedlings in nurseries. The best precaution against this fungus is early spring and less dense sowing. Another important point is to keep the soil reaction between 6.1 and 6.9. For this purpose, the soil to be sown in nursery must be tested before sowing. If it's not proper, then dressing the soil with sulphur or disinfecting the soil with 40 g of 2% sulfuric acid gives good results (Anonymous, 1986; Gezer and Yucedag, 2006).

Perennial rust galls are common on stems and branches of Crimean juniper growing in forests of Isparta and Denizli Regions (A. Lehtijarvi, Isparta, Turkey, personal communication).

However, under suitable site conditions the juniper species grown in Turkey have a good record of being free of diseases and do not cause soil deterioration.

2.2. Seeding Habits

Flowering and Fruiting: Female flowers are seen on two-three years old individuals of Crimean juniper. Male and female flowers usually occur on separate trees in a stand but rarely on same tree. In order to get forest seeds of high quality and quantity, it is important to plant seedlings to produce male and female individuals in seed orchards to be established.

The first crops of viable seeds commonly appear at two to three years and seed production is regular from 20 to 25 years (Gultekin, 2005). Some seed is available every year but good seed crops are produced in two year intervals (Eler and Cetin, 2006).

The number of seeds in a cone, mean seed size and percentage of empty seeds are all positively correlated with size and dry weight of the cones. There is great variation in the germinative power of the seeds related to provenances, cones size, tree age and position of cone in tree crown.

Cone Production: Cone maturation and collection of juniper cones is depending on climate, soil characteristics and physiographical properties of stand site (e.g. altitude, latitude and land exposure of the stands. However, in general seed maturation of Crimean juniper starts in early October and cone harvesting usually starts in March and ends at the last week of April. The mature cones are blackish and bluish in colour. The number of seeds per cone ranges from 3 to 10 seeds.

The harvest of cones is carried out by Directorate of Silviculture and Reforestation groups depending on Conservancies (Directorates of Regional Forests). In practice, the cones are collected by hand (Davis, 1965; Gultekin, 2005).

The collected cones are first spread over the ground as a thin layer for drying. Extraction of the seeds from their fleshy parts is a difficult and tiring process. After this of step process, the dried cones are smashed by wooden mallet and then kept into ashen water about 2-3 day-long periods. At the end of all these of steps processes, the seeds separated from the fleshy parts are washed by clean water under pressure (Gultekin et al., 2005).

There is great variation in germinative power of seeds, and growing characteristics of seedlings related to provenances and the individual genetic characteristics of the trees; moreover, juniper exhibits variations in time of leafing out in spring, leaf regeneration and tolerance to extreme conditions of soils and climate. There is much evidence that the variation is continuous and correlated with environmental factors. In these circumstances, it is very necessary to regulate the transfer of seed from the stand origin to the place of planting. For this purpose, not long ago, a simple zoning system should be prepared.

2.3. Seed Production and Nursery Works

Seed Production: The collection, processing and issue of seeds (and also tree improvement activities) are carried out by "The Directorate of Forest Tree and Seed Improvement Institute", in Turkey.

Twelve Gene Conservation Forests have been selected in natural Juniper forests and registered in management plans by the end of 2005.

The joint characteristics of juniper taxa seeds are the existing physical and physiological germination obstacles. As a physical germination obstacle is the thick and semi-permeable seed coat preventing the penetration and absorption of water and intake of gas (Vanhoverbeke et al., 1985; Rietweld, 1989). Another germination obstacle is the case of embryo premature or some chemical barrier (e.g. chemical inhibitors) existing into the fleshy coat cover of the seed (Yahyaoglu, 1993). These obstacles require certain conditions and pretreatments before seed sowing to eliminate them. For this reason, at the first stage it is required to remove completely the fleshy seed coat. At the second stage it is necessary to apply one or two of pretreatments to the seeds to facilitate the germination of the sound seeds. Some of the pretreatments are: soaking the seed into a sodium solution or boiling water, or treating the seeds by using sulphuric acid, citric acid or hydrogen peroxide with different intensities (Rietweld, 1989). In addition, in order to break the germination obstacles originating from seed coat hardness and embryo dormancy of the species has been a subject for many research scientists (Alpacar, 1988; Avsar and Erenoglu, 2002; Kose, 2000; Gultekin and Ozturk, 2002) in Turkey so far.

In practice, before everything else it is very important to separate the sound seeds from empty ones and eliminate the existing obstacles connect with the physical and physiological properties of the seeds. For this reason, different pretreatments are applied, varying by the juniper species. However, it could be stated here that with the exception of some local studies, there is not enough information about obtaining healthy seeds and also on breaking the dormancy of the seeds of juniper species. The result obtained from the local studies are given below (Table 1).

Table 1. Some Characteristics of Crimean and Phoenician Juniper Seeds and Pretreatment Applied (Gultekin et al., 2005; Gulcu and Gultekin, 2006).

Species	*J. excelsa*	*J. phoenicea*
Amount of seed per kg cones (g)	152	213
Weight of 1000 seeds (g)	25,6	27,5
Proportion of the sound seeds (%)	7	27
Germination frequency (%)	49	71,3
Pretreatments	5 days 20% ashen-water+15 days regular water treatment at 4 °C temperature+ 5 days drying under shade+floating at 25000 ppm NaCl solution+three days keeping in 5000 ppm citric acid+30 days hot-wet creak sand stratification at 15-20 °C temperature+30 days cool-wet stratification in creak sand at 10 °C temperature	Washing with alcohol+3 days 20% ashen-water+3 days 10 000 ppm citric acid+4 hours hydrogen peroxide+5 days regular water treatment combination

Nursery Works: There are 16 state forest nurseries with a total area of nearly to 15-20 ha and a production total capacity of 5 million in Turkey. About 5 million juniper seedlings have been grown at Egirdir Forest Nursery in the year of 2006.

In indicated forest nurseries, Phoenician, Crimean, Odojur and Fruited junipers seedlings have been growing since three years.

For the purpose of producing juniper seedlings, the ground of nursery is mostly ploughed in 30-35 cm depth by 2 or 3 furrow plow (plough) mounted to the medium size (2x4) rubber tyred tractor and then in light compact, the beds are raised by dragging over it heavy wooden sledge or disc-harrow. After leveling, the beds are prepared by means of a bed shaper. Furthermore, the heavy soil is deeply cultivated by using sub-soiler (or ripper-tine) for the purpose of aeration of the soil. Where the soil is very compact, well-rotted manure is advisable. The quantity of well-rotted manure which should be used in varieties from 25 to 30 tons per hectare. Unrotted manure is not used, for the purpose of preventing the young seedlings from damping-off danger. Sometimes, nurse crops like leguminous plants, are grown in the rotation fields of nurseries to improve the soil.

Juniper is extremely easy to rise in nurseries as it is robust and hardy. Seedbeds are prepared in 1.20 m width and 20 cm height, with 20 cm space. The seeds are sown in lines by machines or drills. A seed bed consists of 7 sowing lines with distance of 15 cm intervals.

In general, the proper sowing time of juniper taxa is autumn and winter seasons. It is possible the sowing should be done after early rainfall in autumn. Besides, spring sowing must be avoided from due to late frost (Gultekin and Gultekin, 2005).

The seeds are often treated by using birds repellent before sowing to discourage birds and mice from eating them. For this purpose, an effective chemical mixture is used; the mixture contains two chemical substances named “Pomarsol-forte and alumina dust”. In common use, for 15 kg of Juniper seeds, 800 g Pomarsol-forte and 42 alumine dusts is needed (Anonymous, 1986).

The proper seed sowing depth for Crimean juniper is 4 mm.

Juniper seedlings are fast growing under suitable nursery conditions. Gulcu and Gultekin (2005), indicated that one year old seedlings (1+0) of Crimean juniper could reach up 2,7 mm in collar diameter and 18,7 in height (Gulcu and Gultekin, 2005; Figure 2).

Figure 2. One Years Old of Containerized Crimean Juniper Seedlings (Photo: Yucedag, C., 2006)

It is determined that suitable sowing period of Phoenician juniper under the conditions of Egirdir

Forest Nursery is between the months of July and October (Gultekin et al., 2005).

Seedlings of juniper are easily raised in open nursery beds and can also be raised in politene container or in small plastic tubes the latter being suitable for dry sites but not for mineral soils because of frost lift.

According to traditional application carried out in forest nurseries, after sowing, until germination of seeds, seedbeds should be irrigated for half an hour and once a day, late in the morning; after germination is completed the same procedure should be followed, but this time once in the early morning or late in the evening. When the seedlings are 2 months old, this time, watering is carried out only every other day.

Weeding is usually done by hand or machines; during the period of the first three months. The weeding is made once in three week intervals, and then the weeding is carried out once in five week's intervals. The Turkish foresters are usually not familiar with applying chemical herbicides in forest nurseries.

2.4. Management

Juniper forests of Turkey had been planned and administered in accordance with the diameter class forest management method till the years of 1963. It will be evident that this method is not appropriate for Juniper forests if the stand structure of these forests is examined carefully. Besides, the method is not suitable to reproduce highly intolerant tree species like Juniper. Since 1965 the age class forest management method has been accepted and applied without controversy. Up to 1965, a great part of the forest of juniper were degraded by crude application.

The age class management method is the most suitable one for Juniper forests because of biological and ecological conditions of the species. The age class method takes 20 years for complete regeneration.

Eler (1988) and Carus (2004) indicated that as the junipers are slow growing and poorly suited to measurement methods used for the other tree species of Turkey (e.g. the first reason is wood hardness, heartwood rot which preventing to use the increment borer for determining the age of trees and diameter increment; the second one is the changes in the soil properties by sites, causing difficulties in determination of site classes).

A number of silvicultural systems could be used to regenerate pure stands of juniper in Turkey. The most consistently successful of this has been "The Shelterwood Uniform Method". In this method, the application area is over the entire stand, it is also could be in zones. Cutting is done by leaving shelter trees over the stands or zones.

The rotation ages of Juniper forests are 140 years for good sites, 180 years for medium and poor sites. However, the indicated rotation age could be decreased to the ages of 120-125 years depending on site qualities, because of the heartwood rot starting at these ages.

The height of mature juniper trees in Turkey is 15-20 m and rarely 25 meters. The diameters of up to 80 cm at 1.3 m above ground level have been recorded in the natural Juniper forests in Turkey. The mean annual increment (yield class) is 1.2-3.6 cubic meters per hectare in nature stands. In good sites of Juniper stands, the amount can reach up to 5,2 cubic meters per year. The current annual increment will probably increase to seven cubic meters on using seeds from suitable provenance and applying intensive soil cultivation.

The potential growing stock in middle yield class is 163,9 m^3/ha and the actual growing stock is 19,6 m^3/ha at the age of 100 (Eler, 1988). This result is provided that the differences between the actual and potential growing stock is approximately eight to nine times lower.

2.5. Regeneration Methods

Natural and artificial regeneration activities have not been studied in juniper forests so far. The main reason is lack of scientific information about eco-biological characteristics for proper silvicultural treatment works for the species.

2.5.1. Natural Regenerations

With exception of few local activities towards rehabilitation of juniper stands, the stands have not been widely regenerated by natural regeneration methods in Turkey yet. However, juniper is a light demanding tree and in general successful natural regeneration arises on sides which are open or under a light tree canopy in places which are free of weeds or ground vegetation. In juniper woods regeneration generally arose when an adequate seed supply coincided with good seed years and the absence of heavy grazing (Elicin, 1977).

A number of silvicultural systems have been used to regenerate pure stands of other species in Turkey. However, as the seeds of juniper species are heavy and unwinged, the most consistently successful regeneration method to be used for regenerating pure stands could be shelterwood Uniform System. Regenerations could be greatly stimulated by removal of existing layer of vegetation and exposure of the mineral soil. In dry places a moderate covering of vegetation can be helpful in preventing excessive drying of the soil. Where the regeneration is sparse re-stocking by planting is the best policy.

There are a number of productive stands to be regenerated within the natural distribution of juniper forests. According to the structure of existing the juniper stands in term of their regeneration and tending activities to be applied could be investigated into three groups. These are;

The First group: The density of canopy of stands is more than 40-70 % of the full density and under the stand canopy there are only small herbs and grasses or no weeds. In general, these stands are nearly to over mature age. The stands of this group can be reproduced using the natural regeneration. From personal observations carried out at the end of 2005, it has been determined that there are some successful silvicultural operations towards rehabilitation of Crimean juniper in divisional forest of Sutculer located in Turkey’s Lakes Districts.

The second group: The density of canopy of stands has been much broken and less than 40 % of the full crown density and especially if the stands are situated at south exposures, steep inclinations and at sites near to alpine zones, the soil eroded and deprived of suitable conditions for natural reproduction. However, there are some suitable places for spot (patch) seeding or planting with containerized seedlings. In such as juniper areas, the regeneration of the stands is impossible; the number of seed trees of this stands and their distribution is not homogeneous. The stands of this group can be regenerated only by using seeding or planting. The most of the trees are uneven aged. The proportion of this kind of juniper forest is more than 93% of the total area of juniper forests.

The third group: This group consists of the young natural stands of junipers. Most of these stands are at the age of thinning or tending stage. They are between 40 to 90 years old. The amount of such young stands compromises an area of about 30-40 % of the total area of juniper forests. The thinning and tending operations such as preparatory cuttings for converting the unproductive stands to the stands with normal structure have been delayed and neglected for many years. However, the experiments are in progress, and there are some successful operations in Turkey.

2.5.2. Artificial Regenerations

As it is indicated earlier, the forest land of Juniper approximately covers 0.922 million ha, which comprises about 11% of the total land of coniferous forests in Turkey. According to the latest forest surveys more than half of Juniper forests are at present in degraded conditions. This situation is not only a reflection of ecological condition but also in several areas, a result of illegal or legal overcutting, nomadic grazing and forest fires occurring in the course of several centuries. Juniper stands can be established on about 2 million ha (10%) of the total land of forests in Turkey

After some successful results provided by research studies connecting with production of seeds and seedlings of junipers in a few forest nurseries, it is recommended that degraded juniper stands could be regenerated by seeding. It is possible to put the outcomes provided by findings of recent research studies into practice to rehabilitate the juniper forests. In this connection, planting works have been carried out in some local sites, e.g. Turkey's Lakes Districts and in some parts of the country for last two years. The presented results seem promising for sustainable future of juniper forests. In this contex, from studies carried out within the boundary of Isparta Regional forests, 37 to 48 % of germination is obtained by using spot (patch) seeding and 28 to 39 % of the germinated seedlings overcome summer drought (Gultekin, 2005).

The studies related to growing techniques, classifying of seedling quality (suitable dimensions for planting stock) and afforestration techniques of the species have been continuing together with the other forest activities. In addition, juniper species can also be raised from cuttings. The ability to produce roots declines quickly with increasing age after 5 years. The rooting rate has been estimated to be 31.5 % for Crimean Juniper (Ayan et al., 2004).

3. CONCLUSION

The genus of juniper has a great importance from stand point of Turkey's forestry. This importance comes from the benefits provided by the timber, which has been used in a very wide range from fencing posts through railway sleepers and saw logs for box making, general construction and joinery industries, and wood of some Juniper species is also suitable for naval industry and stationery. Furthermore, some parts of the species play important roles in human health; they are used in chemical and medical industry due to their organic and inorganic components. The species are used to prevent soil erosion and ornamental trees in parks and gardens of the country. However, as a result of centuries long degradation (fires, overgrazing and encroachment for farming, excessive cutting) 93% of the juniper forests are currently under degraded and unproductive conditions and far from providing economical, ecological, social and collective-cultural functions and services expected from them. These degraded and unproductive juniper forest lands are subjected to erosion of different intensities.

In order to protect and provide the sustainability of juniper forests as a genetic heritage, it is necessary to take some measures connecting with rehabilitation of the species. These are;

- The main aim of rehabilitation, which is expected as an important component of protection and sustainable activities, first of all is to win people's confidence and respect for the organization. It is necessary to keep the biological balance of juniper forests for public resources and biodiversity in these areas.

- The unproductive or degraded juniper forest areas should be rehabilitated by means of scientific studies for obtaining the services expected from them. In order to reach the goal, at the first stage it is necessary to designate the exact areas covered by each juniper species. At the second stage, the areas which are mostly suitable for forest establishment should be reforested by the seed sources or seed origins which the suitability are approved by origin trials to the areas to be planted from stand point of establishment ability. In other words, the seed origin to be used should have similar site characteristics (e.g. climate, soil and physiographical conditions) to the areas to be afforested.

- The managing purposes and rotation period of the juniper stands should be determined as to requirements of market and yield classes. For this, it is necessary to define the environmental conditions and structure of the stands. The existing forest division is very large (50-100 ha) for management activities. For facilitating the extensive silvicultural activities, the existing large divisions have to be arranged into small forest sub-divisions (5-20 ha). In addition, it is required to determine the periodical growth and increment of juniper stands.

- Although there are effort and studies on some morphological and physiological characteristics of juniper seeds, further comprehensive scientific studies are needed (e.g. relating to the variation between and within populations).

The other important point to be indicated here is the selection of the proper seed sources for successful reforestation (e.g. seeds must be selected with particular thought to latitude, altitude, climate conditions, soil type and pest resistance). In other words, it is inevitable to compare the productive juniper stands in terms of their seed characteristics in sense of genetic achievement for the future of juniper species.

However, there are a number of bottlenecks to be overcome in the field of tree improvement which are directly relating to the subject of geographic variation, provenance trials of the species and aiming at increasing of seed quality control and certification methods, improvement of the present seed resources and seed stands. Establishment of clonal or seedling orchards as ex-situ is the other major topic that should be taking into consideration.

- Juniper planting stock could also be raised in polythene container or in small plastic tubes the latter being suitable for dry sites but not for mineral soils because of frost lift. Studies concerning growing techniques of the species are being continued in some forest nurseries (e.g. sowing time and depth, seedling density per square meter, root wrenching in bed, lifting and transplanting time, irrigation methods, fertilizing and weeding works etc.)

- The conditions needed for natural establishment of forests are well known but are sometimes difficult to create. However, regeneration could be arisen on sites which are open or under a light crown canopy by means of seeding and planting methods. This kind of activity should be carried out until the scientific research completed. In addition, the extensive afforestation works should be put into practice only in selected plot reforestation areas.

- It is normal practice to fence all plantations to keep out grazing livestock and the other harmful factors and deter villagers living inside or adjacent to the forest from using the juniper areas for agricultural purpose. Besides, compartments in plantation areas are enclosed either with natural breaks, such as streams or ridges, or with cleaned and unplanted breaks or broadleaved belts against fire hazard and probable outbreaks of insects attacks or diseases.

As a conclusion, although there are efforts and studies in order to protect and rehabilitate the existing juniper forests are negatively affected by many threats. Especially the main threats for the areas are local people who are living inside and outside the forests, lack of the institutional structure overusing and other institutional and administrational gaps. Juniper forests have to be protected not only to provide the vital benefits indicated above for human beings such as adequate and clear water for todays but also for the coming generations. For this reason, in order to reduce or keep under control the threats and increase the potential benefits from the juniper ecosystems, we have to support the necessary conservation tools and mechanism. One of these tools and mechanism is to establish a new and special research institute. This prosperous institute would be dealt with solving the problems connecting with seed and seedling characteristics, growing and planting techniques, silvicultural treatments (maintenance, tending, regeneration methods and convertion of the unevenaged stands to evenaged stands), ecological requirements, wood characteristics and uses, causes and damages, growth and yield, variation between and within population of the juniper forests. Thus, it would be a great gift to be sustained this genetic heritage for coming generations.

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Linking the Need for Conservation of Forest Genetic Resources to Requirements of Policy Decision Makers

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Abstract

Genetic diversity harboured in forests is realized to be indispensable for long-term forest sustainability in numerous legally-binding (hard laws) and non-legally binding commitments (soft laws). In recent agreements, resolutions and working programs further and increasing demand to conserve forest genetic resources (FGR) is recognized as new international obligations, scientific findings, changes in the dynamics of forest damage and future threats to the forest (e.g. climate change) need to be considered. In this paper the most relevant hard laws (e.g. Convention on Biological Diversity, Alpine Convention including respective protocols, EU-Directives) and soft laws (e.g. several UN-Declarations, Ministerial Conferences on the Protection of Forests in Europe) are reviewed. However, on the national as well as the international level significant implementation deficits in the conservation of FGR still exist. Despite the fact that the benefits of and need for conservation of diversity in FGR are in general accepted by many policy makers, their practical implementation is frequently difficult, as stakeholders often do not recognize the (economic) importance of FGR conservation. This is especially the case when stakeholders (would) suffer direct financial losses from related measures. The monetary valuation of FGR may help to improve the acceptance for the need of FGR conservation by policy makers and stakeholders and different methods are presented. Further the dialogue between forest geneticists, policy makers and stakeholders needs to be intensified. Three different theoretical approaches – (1) rational choice theory, (2) common property regimes, and (3) advocacy coalition framework – are considered in this paper that may help to reconcile the differing political and economical interests in FGR. These methods may facilitate bridging gaps either by enhancing implementation or picking FGR conservation out as an important topic on the political agenda.

Keywords: forest policy, hard laws, soft laws, stakeholder dialogue

Sixteenth Year Results of a Lebanon Cedar (*Cedrus libani* A. Rich.) Provenance Trial in Central Region of Turkey

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Absract

16 sample plots with totally 35 provenances provided from natural stands of Lebanon Cedar (*Cedrus libani* A. Rich.) were established in Central Anatolia, South-Easter Anatolia, Aegean, Western Mediterranean and Central Black Sea Region at the end of 1989 and at the beginning of 1990. The experiments were laid out in the fields as to ‘‘Randomized Blocks Method ’’ with 3 replications. Each provenance was represented by sixteen 2+0 years old containerized seedling each replications. In this paper, the sixteenth years results of Ankara-İlyakut trial in Central Anatolia were discussed. Seedling survival was observed; total height and diameter at breast height of were measured in order to determine the most successful provenances of *Cedrus libani* A. Rich. And the data were assessed. According to the results; there were considerable differences among provenances for survival (F value Pr<0.005) at age sixteen but provenances were not significantly differ for height (F value Pr<0.09) or diameter (F value Pr<0.25). The most successful sample is numbered as 28 (Saimbeyli-Catak), 22 (Mersin-Arslankoy) and 38 (Ermenek-Kazanci).

Keywords: *Cedrus libani* A. Rich, Provenance Trails

Introduction

Provenance trials consist the most important phase of tree breeding. Provenance trials aim to reveal seed resources, which have high adaptation capability, high genetic heredity and high increment, hence realizing the economic objective best for each region and for each elevation category in forestation and forestation works for a certain species in a certain region. The selection of seed resources is the main factor influencing the productivity of forestation (Ürgenç, S., 1982). Provenance trials will ensure the possibility to determine the best seed resources and will play an important role in the protection of genetic resources of Cedar. *Cedrus libani* seems to be very tolerant tree in forestation areas outside its present natural range. It is one of the most continental tree species in Turkey, able to grow on poor stony, hard and alkaline soils where other species cannot compete with it. During the last 20 years, only 0.07% of the artificial and 1-2 % of the natural regeneration are have been *C.libani*. (Koski, V., Antola, J., 1993).

Material and Method

35 provenances provided from natural stands of Lebanon Cedar (*Cedrus libani* A. Rich.) were established in Central Anatolia (Ankara-Ilyakut trial site) at the end of 1989 and at the beginning of 1990. The experiments were laid out in the fields as to ‘‘Randomized Complete Blocks Method’’ with 3 replications. 16 seedlings in square plots in each block initially represented each

provenance. Spacing was 1.5 m between trees in a row and 3 m between the rows between the rows. Data were collected from Ankara-İlyakut in spring of 2006 when the trees were 16 years old in the field. The data recorded were tree height (cm), diameter at breast height (mm), and the number of surviving individual for each provenance. ANOVA was carried out in order to compare the provenances in terms of height, diameter and surviving rate. “SAS”, statistical analysis program, was used (SAS/stat 1990). Except surviving rate, other variables were analyzed through individuals the former was analyzed through means of parcels. SAS LSMEAN, the least squares mean method, is used in the calculation of means of provenances for height and diameter in an experimental site (SAS, 1990). [E.1] $Yijk = \mu + Bi + Oj + BOij + Eijk$

Results

In the study, mean height values (cm), mean diameter (mm) values and surviving rates (%) in Ankara-Ilyakut experimental site were calculated and given in Table 1 below. There is not significant difference among the provenances for height and diameter at the beginning of 17.th year. Provenances don’t show significant difference in terms of height and diameter. The best heights are 468 cm (Provenance 28, Saimbeyli-Ayvacik), 439 cm (Provenance 22, Arslanköy-Baspinar) and 411 cm (Provenance 38, Ermenek-Kazanci) respectively. The worst heights are 229 cm (Provenance 4, Afyon-Sultandagi), 265 cm (Provenance 18, Kumluca-Yukari Alakir) and 274 cm (Provenance 6, Gölhisar-Dirmil). Provenances show significant difference in surviving rate (56% to 100%). The best survival percentages are 100% (Provenance 19, Elmali-Bucak), 96% (Provenance 23, Ermenek-Dalmacali) and 96% (Provenance 36,K.maras-Elmalar) respectively. The worst survival percentages are 56% (Provenance 33, Niksar-Baraj) and 71% (Provenance 22, Mersin-Arslankoy). Provenances show significant difference in surviving, the fast growing provenances and the ones don’t differ statically are indicated with (*) in Table 1.

There were significant growth differences among the provenances. Up to 35% greater height and 34% greater diameter can be realized at the age of 17, if the best provenances were selected for plantations. A provenance from the fringe distributions of Lebanon Cedar has the worst surviving rate 56%. For this reason, this provenance of Lebanon Cedar should be avoided, but high and middle elevation provenances from Mediterranean region should be preferred in Ankara-Ilyakut experimental site.

Table 1: Diameter (mm), height (cm) and surviving rate (%) least square means of provenances at age 17. Means in bold in a given column are the best performing group.

Provenance Number	Height (cm)	Diameter (mm)	Surviving Rate (%)
2	343	55.8	0.75
4	229	32.9	0.83
6	274	40.0	0.90
9	325	51.2	0.73
10	321	49.7	0.88
11	396	64.5	0.88
12	361	56.1	0.88
13	302	45.6	0.94
14	301	46.6	0.94
15	331	51.3	0.92

16	331	52.6	0.90
17	363	57.8	0.94
18	265	42.4	0.94
19	331	56.7	***1.00**
20	351	55.1	0.94
21	334	50.8	0.92
22	***439**	***73.0**	0.71
23	347	52.1	***0.96**
24	378	61.1	0.92
25	360	55.4	0.90
26	351	56.9	0.73
27	361	58.3	0.90
28	***468**	***74.0**	0.94
29	354	56.3	0.88
30	313	47.8	0.85
31	363	57.6	0.81
32	379	56.7	0.90
33	359	59.1	0.56
34	363	57.9	0.90
35	416	61.7	0.85
36	359	56.2	***0.96**
37	345	54.6	0.92
38	***427**	68.7	0.94
39	347	54.1	0.94
40	338	53.6	0.92
Site Mean	347	55.0	0.87
Pr>F (orijin)	0.0946	0.2494	0.0053

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Somatic Embryogenesis of Forest Trees in Bulgaria: Some Basic Problems & Future Prospects

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Abstract

Alternative strategies in important forest trees improvement program in Bulgaria. Establishment of the embryogenic cultures of *Picea abies* (L.Karst), *Pinus sylvestris* (L.), *Pinus nigra* (Arn.) and *Quercus robur* (L.) is discussed from point of view of the frequency initiation due to the different nutrient and hormonal treatments. The effectiveness of the maintenance of the embryogenic cell lines is determined on the base of the period continuation and the regime during cultivation. The crucial step of maturation and conversion into plantlets are deliberated as a result of the applied media and growth regulators. Possibilities for integration of the cryopreservation and encapsulation techniques in current breeding strategies are also discussed as integral part of the *ex situ* conservation approach.

Keywords: *Pinus, Picea, Quercus,* embryogenesis induction, maturation, germination, cryopreservation

Introduction

Environmental decline due to the current state of the manufactured outputs and the sufficient increase of the consumption of wood, as well as the increasing of negative consequences of this impose new current reassessment of the possibilities of classical propagation methods for the purposes of breeding program in Bulgaria.

Up to day conventional methods of propagating involve the seeds collected from superior seed stands, seed orchard, grafting and rooting of cuttings. The results obtained are not always reliable from the point of deploying improved genetic material. Grafting with the exception of *Abies* hybrids is not economically viable on a large scale. The rooting ability of cuttings taken from mature trees remains behind economically justifiable usage due to the most important hindrance - maturation of tissue as developmental process, which reduced the growth rate and rooting of cuttings. Clonal multiplication of selected superior forest trees is limited to the certain levels in private nurseries and certain species in Bulgaria.

Somatic embryogenesis as an integral part of tissue culture methods can be considered as system with four main stages: induction, proliferation, maturation and conversion into normal plantlets. Each stage requires specific nutritional and hormonal treatments, which have to be finding out mostly experimentally due to well known genetic variation. From the other hand, clonal forestry offers larger estimated genetic gains from selected individuals using sexual offspring (Mullin and Park 1992). In addition, somatic embryogenesis propagation has got two important advantages – production of uniform genetic desirable plant material that can be subjected to cryopreservation and/or production of synthetic seed (Attree and Fowke 1993).

The main goal of the present report is to summarize some basic attempts carried out in Bulgaria to develop laboratory methods for propagation via somatic embryogenesis of economically important forest trees.

Initiation of embryogenic tissue

Several different developmental stages of zygotic embryos of *Picea abies*, *Pinus nigra* and *Pinus sylvestris* were tested for their amenability to produce embryonal-suspensor tissue.

Induction of white, translucent and mucilaginous callus was observed from the micropylar end of *Pinus sylvestris*, *Pinus nigra* and *Picea abies* gametophytes containing immature zygotic embryos (Gjuleva 1998, 2003a,b). The difference observed between species is the windows of embryogenic competence- the third, the fifth and the sixth weeks after fertilization, respectively for all species mentioned above. The callus with the same characteristics can be distinguished when mature zygotic embryos of *Picea abies* were used as initial explants, too. But in the case of usage mature zygotic embryos of *Pinus sylvestris* and *Pinus nigra* as primary explants for all variants tested the formation of embryonal-suspensor tissue was not observed. Both species produced light green solid non-embryogenic callus on the DCR medium containing wide range of 2,4D or NAA and BAP, kinetin or TDZ concentrations up to 10 mg/l in solid or liquid status.

The number of extruded embryogenic tissue varied significantly between and within the species tested. *Pinus sylvestris* and *Pinus nigra* have lower potential to produce embryogenic masses – 0.36% to 9.5% and 0.2% to 16.57% respectively (Gjuleva, 2000, 2003 a,b) in contrast to *Picea abies* – from 27% to 75% registered during experimental work in 2005 for the tenth trees tested (last unpublished results).

When various concentrations of different auxins – 2.4D, NAA and picloram each alone up to 2 mg/l were tested to promote induction of embryogenic tissue from gametophytes belonging to the same mother tree the embryogenic capacity varied significantly, too. In spite of the fact that picloram enriched DCR medium was able to produce up to 9% embryogenic tissue the later growth and development of the proliferating intensively tissue was arrested on medium containing *cis-trans* ±ABA in contrast to the results observed when 2.4D or NAA were used (Gjuleva 2003,b). The same contradictious result considering maturation ability of the proliferated embryogenic tissue was obtained when gametophytes with immature zygotic embryos of *Pinus sylvestris* and mature zygotic embryos of *Picea abies* maintained on half-reduced LM medium were used as initial explants (unpublished results).

Based on the 150 explants tested, the results obtained showed that both maltose and sucrose DCR enriched medium at 20 g/l can be used to initiate embryonal-suspensor tissue from *Pinus sylvestris* and *Pinus nigra* ($\chi^2=0.72<\chi^2=3.84$, df=1 and $\chi^2=1.32<\chi^2=3.84$, df=1) respectively. The efficiency of glucose was still low from the point of view of the lower number of extruded embryogenic tissue and therefore was not suitable for further usage (Gjuleva, 2000, 2003a).

Completely different hormonal and nutritional regime was developed for *Quercus robur* to provoke expression of embryogenic response of tissue. The best embryogenic response 94.4%

and 86.1% was registered for donor tree #1 and #4 respectively on MS medium supplemented with either 1.0 mg/l BAP alone or in combination with 1.0 mg/l GA_3. The embryos collected during the first week of July demonstrated the highest competence that rapidly decreased and got lost by the end of the period tested (Tzvetkov,1998) although TDZ could induce the initiation of the process in common oak immature embryos in very low doses (Tzvetkov, 1999, 2004). The results obtained showed that the highest embryogenic response was observed in using fructose. The relationships between the carbohydrate concentration and frequency of the embryogenic response followed quadratic (sucrose and fructose) and cubic (glucose) regression lines, respectively. The predicted values of optimal embryogenic response in respect of carbohydrate levels varied between 30g/l and 60 g/l.

In vitro regeneration from internodal segments of *Sorbus domestica* was found out but somatic embryogenesis was not observed on the variants tested (Tzvetkov, 2003).

Maintenance of embryogenic tissue for prolonged time

Maintenance of proliferating embryogenic masses of all coniferous species studied and *Quercus robur* was achieved for several years (Gjuleva 1998, 2000, 2003 a,b, Tzvetkov 1998, Tzvetkov and Gjuleva 2001). The serious hindrance is the decline in embryogenic competence and/or the lack of response to ABA treatment. In fact, from the point of view of retaining of higher maturation ability the results on *Picea abies* embryogenic cultures clearly showed that prolonged proliferation up to 4 months does not arrest later maturation capacity on medium containing *cis-trans* ±ABA of type A embryogenic tissue (Gjuleva, von Arnold 1999). The results obtained when the embryogenic cultures of *Pinus sylvestris* and *Pinus nigra* were subjected to *cis-trans* ±ABA to produce somatic embryos showed that the longer maintained proliferation provoke embryogenic pine cell lines cultures to lose their proliferation and became brown. Only separate brown nodules scattered on the surface of embryogenic clusters retained the capacity to reproliferate when they cultured on fresh induction medium. The detailed results showed that only 15% of the initially initiated embryogenic cell lines of *Picea abies* retained steady capacity to proliferate after six months on proliferation medium. Similar results were observed with the embryogenic cell lines of *Pinus nigra* and *Pinus sylvestris*. According to Tzvetkov (1998) embryogenic cultures of *Quercus robur* could be easily maintained in darkness by regular transfers on hormone-free MS medium without loss of embryogenic potential but no investigations are carried out to show how potentially the maturation ability and conversion into normal plantlets will be changed.

Cryopreservation procedure of the proliferating embryogenic tissue could be the best decision for overcoming of apparently undergoing aging process or decline of embryogenic lines in a view of published protocols (Haggman et al, 1998) but getting support in equipment and consumes for such studies is rather hard at least for now in Bulgaria.

Maturation and conversion into normal plants

During 10 recent years several intensive studies were conducted in order to bring light on the most crucial stage of somatic embryogenesis, namely maturation and conversion into plantlets. The focus was placed on the study different effect of ABA, PEG_{4000}, phytagel and carbohydrate sources – maltose, glucose, lactose and sucrose in various concentrations.

On the medium enriched with 80μM -100μM *cis-trans* ±ABA and 40-60 μM *cis-trans* ±ABA respectively and/or PEG_{4000} embryogenic clusters of *Pinus nigra* and *Pinus sylvestris* were able to produce mature somatic embryos but statistically significant variation was obtained between and within cell lines tested. The arrest in the development of mature somatic embryos of both *Pinus sylvestris* and *Pinus nigra* was observed (Gjuleva, 2000; Tzvetkov, Gjuleva 2001). The replacement of the PEG_{4000} with 9 g/l Phytagel in the maturation medium led to the better expression of maturation capacity and formation of well-defined (shoot/root meristem tissue) mature somatic embryos (Gjuleva 2003 a,b). The best results considering conversion capacity into normal plantlets were achieved when only three months proliferating embryogenic clusters were used for both pine species and only type I mature somatic embryos were used. Histological examination of such mature somatic embryos of coniferous studied revealed that both shoot and root meristem and procambial tissue are well defined (Gjuleva and von Arnold,1999 Gjuleva 2003a,b).

Some common oak mature embryos successfully germinated after being transferred to WPM medium with 0.2 mg/l BAP (Tzvetkov,1998). The results obtained from the experiments on maturation stage of *Quercus robur* showed that both exposure time of ABA treatment and genotype of the embryogenic cell line influenced rather significantly the number of the cotyledonary embryos produced. The significant interaction was observed between genotype and ABA concentrations. Moreover, the maltose was proved to be inappropriate carbohydrate source for maturation of common oak somatic embryos (Tzvetkov, Gjuleva 2001).

The results on *conversion* into plantlets of *Picea abies* type I mature somatic embryos obtained showed that the adding of active charcoal to the germination medium joined with IBA and /or NAA had significant effect on the following development of both shoots and roots simultaneously ($t_{exp.}=6.196$, df=38 and p=0.0003). Lower results were obtained when GA_3 in various concentration were tested in order to produce *in vitro* seedlings.

All coniferous species studied grew successfully on the commercially developed substrate in the green house for one season after being adapted for three weeks under high humidity.

Some embryogenic masses plated on WPM medium with 0.2 mg/l BAP produced somatic embryos. Although the majority of embryos failed to mature, few of them reached a later late cotyledonary-stage and successfully germinated (Tzvetkov 1998, 2004b).

As a result of the carried out experiments during recent years the development of the basic methodological aspects were established in regard of somatic embryogenesis of important for forest practice in Bulgaria species.

Cryopreservation and synthetic seed and/or plants production

Cryopreservation or conservation at -196^0C in liquid nitrogen (LN) demonstrate the feasibility of long-term conservation of valuable germplasm. Different successful attempts have been accomplished demonstrating the power of this approach.

The gradually decrease of the viability of germplasm of forest trees conserved in traditional seedbanks and the problems associated with the collection and multiplication enforced the usage of alternative storage methods either way seeds or zygotic embryos and/or embryogenic tissue or somatic embryos.

Pita et al. (1997) studied the effect of cryopreservation on the viability of *Pinus nigra* and *Pinus sylvestris* seeds. The results showed that germination is not significantly different in any of the blocks included in their experimental design (*cryopreservation* x *desiccation*). It was suggested that cryopreservation could be an economical and practical method in the conservation of seeds of *Pinus* species.

Moreover, *Haggman* et al. (1998) developed an effective cryopreservation method for Scots pine (*Pinus sylvestris* L.) embryogenic cultures. Altogether nine cell lines derived from three mother trees were cryopreserved after cold hardening using dimethylsulfoxide or two different mixtures of polyethyleneglycol 6000, glucose and dimethylsulfoxide as cryoprotectants. Most of the cell lines remained viable after cryostorage with the best cryoprotectant treatment being 10% PEG_{6000}, 10% glucose, and 10% DMSO in water. This treatment resulted in significantly better regrowth of the embryogenic cultures than with the other cryoprotectants or with the controls. When proliferation growth of the frozen cultures had started, their morphological appearance was the same as the non-frozen cultures. In addition, the RAPD assays suggested that the cryostorage treatment used preserved the genetic fidelity of the Scots pine embryogenic cultures. *Ford* et al. (2000) in an attempt to improve and optimize the protocol to cryopreserve *Pinus patula* embryogenic tissue described two aspects that influence tissue recovery after cryopreservation: (i) the effects of precooling tissue prior to immersion into LN; and (ii) whether the choice of supports onto which the recovered tissue is suspended improved the recovery rate. Results indicated that precooling tissue to -70°C prior to immersion into LN was superior. Tissue recovery improved when polyester grids were used as supports.

Alternative way to preserve valuable germplasm is the application of artificial seed technology. *Sparg* et al. (2002) used somatic and zygotic embryos of *Pinus patula* for encapsulation in 2.2% sodium alginate to examine the potential of producing artificial seeds. Somatic embryos encapsulated in alginate beads supplemented with both sucrose and charcoal germinated from the alginate beads after 14 days, and could be stored for 20 days with a 30% decrease in the percentage germination. *Malabadi* et al. (2005) proved that the germination percentage of encapsulated somatic embryos was affected significantly by the concentration of sodium alginate and the duration of exposure to calcium chloride. Somatic embryos encapsulated with 2.5% sodium alginate dissolved in DCR basal salts gave significantly higher germination (89%) than other treatments. Short (5 min) incubation of the alginate encapsulated embryos in calcium chloride solution proved to be the best encapsulation procedure and the embryos subsequently gave the highest germination (89%). Synthetic seeds could be stored at 2 °C for 120 days without a reduction in germination as opposed to non-encapsulated somatic embryos which showed only 9% germination after 20 days at 2 °C. Germinated synthetic seeds produced normal plantlets.

In Bulgaria, investigations on conservation of different tissue and organs of forest trees in liquid nitrogen are not carried out mainly due to serious economical difficulties. However, some factors found to influence in a positive way regrowth after encapsulation of nodal segments of *Quercus robur* and *Ulmus laevis* were determined (Tzvetkov, Hausman, 2004; Gjuleva, Stojanov, 2004). The positive results obtained with other forest trees are the good basis for the integration of pines, spruce and common oak somatic embryogenesis in the cryopreservation and/or encapsulation project as an integral part of contemporary *ex situ* conservation strategy.

Conclusion and future prospects

Tissue culture technology can be the powerful tool for supplying of large numbers of planting material for forestry but somatic embryogenesis still meet several limitations such as sufficient genotype effect, limited number of explants amenable to produce embryogenic masses – especially valid for *Pinus sylvestris* and *Pinus nigra* in contrast to *Picea abies*, lost of the embryogenic competence after long-term proliferation, strong variation of cell lines on ABA containing medium and still non high enough conversion into normal plants. For the first time in Bulgaria the integration of somatic embryogenesis of forest trees to the contemporary breeding program in Bulgaria was suggested (Tzvetkov and Gjuleva 2001).

One of the great advantages of the somatic embryogenesis is the opportunity for cryo-storage of superior forest tree germplasm and/or encapsulation technique that can be served different purposes of *ex situ* conservation strategy in Bulgaria.

Further more investigations are needed at each stage for improving the cultivation *in vitro* response joint with concomitant both biochemical and molecular research for better understanding the mechanism of induction, proliferation, maturation and conversion into normal plantlets. The last is important in order to the genetic fidelity to be maintained on the base of reliable molecular markers at the early juvenile stage of plant development.

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Low-cost Propagation Techniques for *Bambusa vulgaris Schrad ex wendl* Through Branch Cutting

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Abstract

A low-cost propagation trial was carried out over a period of two years from July 2004 to June 2006 to explore the clonal propagation potential of *Bambusa vulgaris* Schrad ex wendl with four different types of branch cuttings (base cuttings, secondary branch cuttings, nodal leafy cuttings and tip cuttings) collected from six years old clamps. To assess the rooting ability of cuttings, they were treated with 0%, 0.1%, 0.4% and 0.8% IBA solutions and kept in the open place (for base cuttings and secondary branch cuttings) or in the non-mist propagator (for nodal leafy cuttings and tip cuttings) for letting them root. The cuttings rooted six weeks after setting in the propagation medium and were allowed to grow in the polybags for ten months under nursery condition for assessing the steckling capacity. Finally, the propagules were planted in the field for field performance. The study revealed that all types of branch cuttings of the species were able to develop roots, shoots, to survive and form rhizome in the nursery and field condition. Rooting ability of the cuttings was significantly enhanced due to the application of rooting hormone IBA. The highest rooting percentage in base cuttings, nodal leafy cuttings and the tip cuttings (60.0, 56.67 and 51.0 respectively) were observed in 0.8% IBA treatment followed by 0.4% IBA treatment and the lowest (36.67, 34.3 and 30.0 respectively) was in the controlled cuttings. However, in the secondary branch cuttings, highest rooting percentage was 72 in the 0.4% IBA treated cuttings followed by a rooting percentage of 66 in 0.8% IBA treatment and the lowest was 42.33 in the cuttings of without treatment. The highest number of roots developed per cutting (9.42, 8.1, 9.77 and 8.33 in base cuttings, secondary cuttings, nodal leafy cuttings and the tip cuttings respectively) was obtained in the cuttings treated with 0.8% IBA solution followed 0.4% IBA treatment and the lowest (6.3, 4.2, 3.1 and 2.1 respectively) was in the cuttings without treatment across the cutting types. However, the length of the longest root varied significantly neither with the cutting types nor with the intensity of IBA solution. Survival percentage of the stecklings was significantly enhanced by the applied rooting hormone in nursery condition but was indifferent in the plantation. The result of the present study was explained in the content of cutting yield, rooting ability, steckling capacity and the field performance of the rooted cuttings for mass clonal propagation.

Keywords: *Bambusa vulgaris*, non-mist propagator, rooting ability, steckling capacity.

The Role of Cloning in Low Input Breeding and Testing of Forest Tree Species

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Abstract

In progeny testing of forest tree improvement programs, family means are estimated with certain precision by sampling environmental variance with a large number of progeny. However, within family selections are based on phenotypes that are not efficient. We explored efficiency of cloned versus seedling progeny testing for within-family selection. Cloned progeny testing proved greater efficiency in sampling micro environmental variation than seedling progeny of the same full-sib families. Consistent smaller within-plot variances were reported from rooted cuttings than seedlings of the same full-sib families. Better sampling of environmental variance by clones reflected in higher precision of additive genetic variance estimation with smaller standard errors. Similarly, greater family and within-family heritabilities were estimated from cloned progeny than seedlings. Full-sib mean heritability for 6-year volume from clones (~0.62) was about two times greater than heritability (~0.30) from seedlings. We found that, even when genetic gains from cloned family and within-family selection were adjusted for the cost (number of trees tested) and for longer testing cycles, greater genetic gain could be realized than seedling progeny testing. In conclusion, cloned progeny trials should be considered to increase the efficiency of current and future breeding programs of forest trees.

Key words: Progeny testing, additive genetic variance, breeding, selection, breeding value

Conversion of a Progeny Trial of *Eucalyptus tereticornis* to a Seedling Seed Orchard Considering Gain and Fertility

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Abstract

An open pollinated progeny trial of fifty families of *E. tereticornis* was evaluated at age four. It was thinned to 625 of initially planted 1250 trees by truncation selection for the phenotype (an index weighting the tree values for height, stem diameter and form). The remaining trees were evaluated for fertility at age seven. Only 21% of the trees were fertile. Combined index selection and phenotypic selection based on the growth data at age 4 were compared assuming an additional thinning down to 200. Gain and diversity of the predicted crop was calculated based on the fertility registrations. Combined index selection gave 5% less gain than phenotypic selection, and 25% reduction in diversity (status number). Sibling coefficient (measure of fertility variation among the retained trees) was 39% higher with index selection. Thus selection for breeding value (gain) seemed less efficient than phenotypic selection for this particular case of converting a progeny test plantation to a seedling seed orchard. Since fertility is low in first generation introductions of *E. tereticornis* in moist tropical regions, improvement in fertility is likely to reduce inbreeding in offspring and promote representation from more families.

Keywords: selection, gene diversity, status number, genetic thinning

Introduction

Seedling seed orchards are commonly used as production populations in breeding programs for short rotation tropical eucalypts (Eldridge *et al*., 1993). As land races suffer from hybrid breakdown and narrow genetic base (Davidson, 1998; Boland, 1981), open pollinated families of selected natural provenances are generally used to initiate the program. As a short term strategy for meeting the immediate seed requirement, fifty good *E. tereticornis* trees selected in existing first generation introductions from Australia were used for establishing a progeny trial and conversion to seedling seed orchard after evaluation and thinning (Varghese *et al.* 2001). Seedling orchards are expected to generate superior quality seed compared to other unimproved sources. Selection methods for increasing gain are often opposed to strategies for improving diversity of the seed crop. Different selection strategies can be employed for enhancing gain. In combined index selection weighting is given based on individual and family values (Falconer and Mackay, 1996) whereas phenotypic selection ranks the individual based on its phenotypic value without considering its family merit. An ideal strategy would be one that combines gain and diversity monitored in terms of a quantified effective population number (Lindgren *et al*., 1996) with adequate representation from different families for production of out crossed seed. When thinning done in a half sib progeny trial, the relationship between genetic gain and diversity must be well understood. These factors must be predicted to adequately manage inbreeding in plantations and to plan seed collection. Seedling seed orchards are expected to serve as a means of packaging genes and generate improved seed at the culmination of a generation of breeding. Breeding values are normally used for culling the inferior families and individuals to maximise gain. Fertility of trees also has to be considered as it ultimately decides the transfer of the genes to the seed crop. The quality of seed output from an orchard will be judged from the possible gain and the diversity of the deployed crop. Genetic value of seed will be determined by the breeding values and the maternal and paternal gametes produced by the orchard trees. This study aims to predict the impact of a hypothetical selection thinning schedule on the genetic gain and diversity in a second generation progeny trial of *E. tereticornis*..

Material

A progeny trial of *Eucalyptus tereticornis* was established at Tamil nadu (10° 23'N latitude, 78° 49'E longitude, 180m altitude, 650mm rainfall) in southern India. The trial comprised 50 families originating from open pollination of selections in first generation Indian plantations. The initial spacing was 2m x 1m. The trial was evaluated at age four. The progeny trial was thinned to half of initially planted by truncation selection for an index weighting the phenotype for tree height, stem diameter and tree form. The remaining trees were evaluated for fertility at age seven. Observation of the number of flowers and fruits on each tree were used as measures of female and male fertilities respectively. The trial was to be converted to a seedling seed orchard after a second thinning, as part of a breeding program for eucalypts (Doran *et al.* 1996). Predictions of breeding values of individual trees for tree height were obtained after evaluating the trial at four years of age as a combined index of maternal family and the individual phenotype = breeding value (Falconer and Mackay, 1996). A hypothetical selection of the 200 best trees was done either by combined index selection or phenotypic selection using select function of the Data Plus program (Williams *et al.* 1999).

Theory and methods

The theoretical background for seed orchards utilized in this study was developed by Lindgren and Mullin (1998). Group coancestry (Θ) is the probability that two genes taken at random from the gene pool of the expected seed orchard crop will be identical by descent. Group coancestry applied for a diploid population is the average of all coancestry values between population members (including self-coancestry), but as group coancestry depends only on the gene pool and not how it is organized into individuals, the group coancestry concept can as well be applied to successful gametes before they form diploid zygotes. Status number (N_s) is the number of non inbred non related genotypes sampled from the reference population that are expected to experience the same deviation in gene frequencies from the reference population as the population under study. These two parameters are used to monitor the diversity level in the orchard resulting from relatedness of selected trees. Sibling coefficient (ψ), which is the probability that two genes originate from the same parent, is used to quantify the extent of fertility differences between orchard genotypes. Maternal and paternal fertilities of each tree are used as the probability of contribution of each genotype in determining the group coancestry (Θ), which can be obtained by adding all possible pairings of gametes from orchard trees.

Flower and fruit production in the trees were recorded during the period October 2005 to January 2006. The number of primary, secondary and tertiary branches was counted in each tree and the flowers per tertiary branch recorded for all the trees during two visits in the peak flowering period of October - November. The number of developed fruits per secondary branch was recorded in two visits during the period of seed collection (December - January). Estimates of number of fruits and stamens per tree were obtained by extrapolating the counts made on stamens and fruits (Bila *et al.* 1999; Kang and Lindgren 1998).

Genetic gain - was worked out as the deviation of the average breeding value of the selected tree from the mean breeding value of all trees measured at year 4 (Lindgren *et al.*, 1989) and the fertility contribution using the formula

$$G = \sum_{i=1}^{N} p_i (g_i - g_a)$$

where g_i is the breeding value of the i^{th} selected tree and g_a the mean breeding value of all (N) trees and p_i the proportion of fertility of the i^{th} tree .

Group coancestry can be divided in two (additive) terms, self-coancesty (Θ_s) and paircoancestry (Θ_p).Group coancestry of the gametes in the orchards indicates the coancestry that the seeds originate from the same parents as eucalyptus has a mixed mating system. Pair group coancestry indicates the group coancestry of the seeds originating from related trees. This causes inbreeding in seeds during the mating. Summation over other trees from the same family was done to consider the contribution from half sibs to the expected group coancestry of the expected crop, $p_i p_j$ when i and j belongs to the same family.

$$\Theta_p = 0.125 \sum_{i=1}^{N} \sum_{j=1 if j and i}^{N} p_i p_j \quad _{belongtosamefamily}$$

Status number (N_S): The diversity of the expected seed crop can expressed in terms of group coancestry as a type of effective number, the status number

$$N_s = \frac{0.5}{\Theta}$$

Relative status number: The relative status number ($N_r = N_s / N$, where N is the census number of selected trees) was worked out to compare the effective number of trees with the respective census number

Sibling coefficient (Ψ) : It is calculated from the number of trees in the orchard (N) and individual fertility (p_i) of each tree and used to describe fertility variation among the trees.

$$\psi = N\sum_{i=1}^{N} p_i^2$$

Gene diversity

Expected gene diversity (*GD*) is a function of the group coancestry and can be calculated in the orchard relative to a reference population. Since the reference population (which is the natural forest the plus trees were selected from) has zero group coancestry, gene diversity is calculated based on the increase in Θ since breeding operations started.

$$GD = 1 - \Theta$$

Results and Discussion

The results are presented in Table 1 below.

The major considerations in a selection program are selection intensity, genetic gain, inbreeding and gene diversity. For a given intensity of selection, there has to be a compromise between gain and diversity as the two effects operate in conflict with each other. Generally the prime objective of any improvement program would be to increase gain in the next generation. Genetic diversity in the orchard is however necessary for production of vigorous progeny in species with mixed mating system and number of represented families can have a great impact on the structure of the seed crop. The inbreeding that sets in is a function of the coancestry of the orchard parents, which could restrict the future improvement possible. Gene diversity can also have a value for the productivity of a stand, it can be a safe guard against calamities, and it is expected that a diverse crop utilize the site better. The impact of all these factors has to be considered when thinning is planned in the orchard.

Combined index selection, which gives emphasis to family performance, had trees from fewer families represented compared to the other two methods. Phenotypic selection which is essentially a truncation of outstanding phenotypes resulted in greater number of families being represented at the same selection intensity.

Genetic gain

Genetic gain (computed as the product of the index value and the fertility contribution) was 5% lower in index selection since the selections were from superior families where many trees were not fertile (Table 1). In phenotypic selection, phenotypically outstanding individuals from inferior families were also represented giving lower breeding values but since many trees were fertile the average gain was high. Index selection had 21% less number of families represented than phenotypic selection.

Fertility status

Only 21% of the trees were fertile in the stand at seven years. The situation appears slightly improved in this second generation orchard compared to an earlier study in a first generation orchard of *Eucalyptus tereticornis* (Varghese *et al*. 2002) where only 17.5% of the 200 selected trees were fertile resulting in high fertility variation ($\Psi = 17.4$). Sibling coefficient value in this study is considerably lower than that in the first generation orchard. It is however 28% lower for phenotypic selection than for index selection. It is still considerably higher than the predicted value ($\Psi = 2.62$) for seedling seed orchards of hardwood species (Kang *et al*. 2003). Thus fertility variation in *E. tereticornis* may be regarded as unusually high in tropical regions (Pinyopusarerk and Harwood, 2003) and due consideration should be given to this factor when planning orchards for seed production.

Diversity

Loss of diversity and increase in relatedness are expected in the advanced stages of improvement in a species. Improvement in gain is at the cost of diversity with each advance in generation. It is however important to monitor the consequences of selection and thinning done to enhance gain. As relatedness increase beyond a certain point, depending on the deviation from random mating, much of the desired benefits may not be achieved. Kang *et al*. (2001) recommended an effective clone number of at least 10 in an advanced generation clonal seed orchard with an equal number of ramets per clone. It is desirable to keep the levels of coancestry low when fertility variation is high and a strategy of retaining fertile phenotypically superior trees can enhance the effective population size by 33% than with index selection.

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Table1. Estimate of gain and diversity with different selection strategies

Parameter	Combined Index selection	Phenotypic selection
Number of trees (N)	200	200
Number of families	37	47
Group coancestry (Θ)	0.0410	0.0307
Status number (N_s)	12.2	16.29
Sibling coefficient (Ψ)	13.96	10.06
Relative status number (N_r)	0.061	0.081
Gene diversity (GD)	0.9590	0.9693
Gain (G)	92.12	96.67

A Low Input For High Diversity Conservation Program in Turkey

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Abstract

Turkey offers vast diversity in climate and ecosystems which have been reflected in the presence of diverse floristic regions, diversity centers of cultivated plants and great diversity in biodiversity. There are over 10 000 plant species and one-third of these are considered to be endemic to the country. Gymnosperms, alone, are represented with 23 species, three of these being endemic.

Turkey has well established legal grounds and organizations to establish and manage conservation programs. T*urkish Constitution, National Parks Law, Forestry Law, and Pasture law* provide legal basis for government agencies mainly within the Ministry of Environment and Forest (MoEF) to implement and manage the conservation programs. Especially, The General Directorate of Nature Conservation and National Parks (GDNCNP) of MoEF are responsible of management of general conservation programs such as national parks (NP), nature conservation areas (NCA), nature parks and wildlife conservation areas. To date, 35 NP (covering 856.678 ha), 35 NCA (84 230 ha) and 17 nature parks (69 370 ha) have been set up (*http://www.cevreorman.gov.tr*). There are also other less strictly protected conservation programs which are managed by other government agencies such as *Specially Protected Environments*, *State Farms* and *Protection Forests*. These various programs made up little over 5% of the country. The ultimate goal is to reach 10% of the country, setting up as protected areas (Kaya and Raynal, 2001).

Biodiversity richness of the country was also evident in forest genetic resources. There are over 50 tree species that could be commercially exploited. However, conservation and breeding programs, due to their high costs and institutional capacity of the Turkish Forest Tree and Seeds Breeding Directorate (TFTSBRD) , have been focused on the priority species indicated in the *National Tree Breeding and Seed Producttion Program* drafted in 1994. The conservation programs are mainly gene conervation forests (GCF), seed stands (SS), seed orchard (SO) and clone banks (CB), established and managed by the TFTSBRD with collaboration with General Directorate of Forest. The *Gene Management Zones* program is a recent one and limited two pilot sites(Bolkar and Kaz Mountains) at the moment. To date , 162 GCFs for 23 , 344 SS for 27 , 166 clonal for 11 and 35 seedling seed orchards for 19 and 10 clone banks for 5 tree species have been set up(*http://www.ortohum.gov.tr*). The number of GCFs for commercially important tree species is expected to increase in coming years.

To set up conservation programs do not guarantee to have an efficient biodiversity conservation since in addition to inadequacies in financial sources, public awareness, participatory management and institutional capacity, there are many social and economical problems of

biodiversity conservation in Turkey. Some of these could be listed as population growth, traditional agriculture and animal husbandary, over exploitation of resources, forest fires, low income people living close to natural resources, unregulated and over grazing of meadows and pastures, habitat destructions due to industrilization and urbanization, toursitic developments, soil erosion and floods and inproper use of land and lack of land use classification (Kaya, 2004). The signing of *Convention on Biological Diversity* (1992) and ratification in 1994 by Turkey provided driving force to tackle the problems of biodiversity conservation. Thus, The *National Biodiversity Strategies and Action Plan, National Environmental Strategy and Action Plan and National Action Plan for Combatting with Desertification* were prepared by the government agencies. To meet the committements in these action plans and the needs for nationally effective biodiversity conservation programs, two Global Environmental Facility fund of the World bank funded projects (*In-Situ Conservation of Genetic Resources* (1993-1999) and *Biodiversity and Natural Resource Management Projects* (2000-2007)) were carried out in Turkey.

In-Situ Conservation of Genetic Resources (1993-1999) (GEF-1) were collaboratively realized by the Ministries of Agriculture, Environment, and Forest and yielded the development of Gene management zone (GMZ) concept, education of staff from Ministries of Forestry, Agriculture, and Environment on gene conservation, GMZ development in pilot sites, public awarness and participation, collaboration among government agencies, international symposium on *in situ* conservation and *National Plan for in situ Conservation of Plant GeneticDiversity*. As a continuation of GEF-1, Biodiversity and Natural Resource Management Projects (2000-2007) (GEF-2) were initiated. Funding from this project helped to improve institutional capacity and technical trainings, preparation of legal documents such as national plans, action plans, new forest management regulations, new protected area management and protected area-network programs for sustainable *in situ* conservation of plant genetic resources and biological diversity.

The TFTSBRD should continue to have intensive conservation (clone bank, seed orchards) and breeding programs in those species listed in the *National Tree Breeding Program* as priority species such as *Pinus nigra, Pinus brutia, Cedrus libani, Pinus sylvestris* and *Fagus oriantalis.* But for other forest trees, to have low input and efficient gene conservation in forest trees, seeds stands and gene conservation forests should be integrated nationally with forest management plans and protected area-network since nationwide biodiversity conservation programs such as NPs and NCAs are managed and monitored by GDNCNP which has better institutional and funding capacity than TFTSBRD.

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The Importance of Igneada Floodplain Forests in Terms of Nature Conservation and Genetic Diversity

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Abstract

Igneada region is very valuable, in terms of ecosystem diversity. There are a lot of vegetation types in Igneada region like, flooded forests, high forests around the flooded forests, lakes some of which are seasonaly linked with Black Sea, swamps around the lakes and a large sand dune. The ecological, biological, environmental and economical importance of the wetlands and floodplain forests has been appeared and irregular use of these environments for centuries makes them more important. As a result of the irregular use, the covarage of the floodplain forests in Europe has been decreased. On the other hand, a flooded forest as a forest reserve is one of the most important components to identify the biodiversity. There are three floodplain forests in Iğneada one of which is a natural conservation area. As a result of the intensive forestry, grazing and illegal cutting, the structure of the floodplain forests in Igneada has been particularly damaged. Main tree species of the forests are; *Fraxinus angustifolia*, *Alnus glutinosa*, *Quercus robur*, *Carpinus betulus*, *Juglans regia*, *Acer campestre*, *Acer trautwetteri*, *Ulmus laevis* and *Ulmus minör.* The distribution of these species mostly depends on the soil conditions as a result of their limited ecological amplitudes. Because of their reduced habitat demands they couldn't largely distribute like *Quercus frainetto*, *Q.petraea*, *Fagus orientalis*, *Pinus* species etc. On the other hand, in Igneada Floodplain Forests there is neither any genetic linkage nor any ecological similarity with other floodplain forests due to the geographical position and isolation. This phenomenon makes Igneada Floodplain Forests more important and sensible according to ecological and genetically point of views. Because of this, Iğneada Floodplain Forests and its province have to be reserved as a natural conservation area. Beside this, local seed must be used in the reforestation activities both on riparian and plain places around Igneada.

Keywords: Nature conservancy, genetic diversity, biodiversity, *Fraxinus angustifolia*

Application of low input breeding strategies for tree improvement in Nepal

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1. The Nepalese context

The Nepalese context in relation to breeding for improved farmland trees may be simplified into eight headings (based on Lillesø et al. 2001a):
(1) A large number of trees are utilised by rural small holders in Nepal for multiple purposes and in multiple ecological zones. Well-adapted seed sources may ensure a reliable yield for farmers, while maladapted seed sources may result in loss. The size of such loss or 'values forgone' by applying non adapted seed sources has not been established at present, but a risk for large losses obviously exist, (2) Trees provide the rural communities with wooden products, serve as a cash crop and provide jobs in local sawmills and small manufacturing industries. This is especially the case in the low land where transport is less complicated, (3) Trees provide fodder that is of crucial importance for cattle feeding, and - through manure and bedding – provide a key input of nutrients to the agricultural system. Improved trees in the farmlands are a key to improved agriculture. Also, fruits are of crucial importance for human nutrition. (4) Many fruit trees have complicated pollination systems, scattered distributions in the farmland and are often propagated from vegetative propagation, (5) Extraordinary large ecological variation exists within small geographic distances in Nepal. The distribution and production of species varies drastically with the extreme topographic variation in the country (from alpine to tropical). Special attention must thus be given to ecological considerations, and GxE interactions may reduce gain substantially for many species unless improvement is performed according to a multiple breeding populations approach, (6) Infrastructure is poor in large parts of Nepal. Travel by foot is important and movement of seedlings often limited to short distances. Delivery programmes are most likely to function if based on decentralised propagation, (7) Experience with tree improvement in Nepal was very limited prior to the initiation of the tree improvement programmes in 1994, and training and capacity building were required, (8) Tree seed is not traded on effective markets. Structures for exchange of seed and information are lacking and this increases the need for integrating considerations for delivery systems when designing the improvement strategy.

2 Tree plantings in the heterogeneous landscapes

Most farmland tree species grown for fodder and fruits are relatively fast growing, but their genetic potential is under-utilised as largely undomesticated varieties are grown. It is well known that improved growth, quality and adaptation can be achieved by carefully selecting the best seed sources when raising seedlings for a given planting (Foster *et al.* 1995). Genetic potential can therefore be released to the benefit of rural people, if a proper species-site and seed sources-site matching is performed, and if the species are brought into a careful (but unsophisticated) domestication process. A Tree Improvement and Silviculture Centre (TISC) has been formed in Nepal to meet the challenge of implementing a programme for breeding and delivery of improved planting material to the benefit of rural small holders.

An important step in implementation of the breeding and delivery programme was development of a Tree Planting Zones system based on ecological mapping (Lillesø *et al*. 2001b). This was done in the form of an ArcInfo data base that combines available GIS data with information from available vegetation surveys and existing vegetation maps (Lillesø *et al*. 2005). The planting zone system is intended for delineation of both breeding and deployment zones. Planting sites with similar environmental conditions are thus grouped together into zones (in some cases a given tree planting zone may consist of several physically separate areas with similar ecological conditions). The idea is that specific seed sources can be developed for – and applied in - a given planting zone increasing the likelihood of fast growing, healthy trees. The system of "Tree Planting Zones" is thus intended to become part of a tree seed distribution system aiming at contributing to improved living conditions for the rural small holders in Nepal, even for species that has not yet been improved through selection and breeding.

3 Species selected for domestication though genetic mobilisation and seed source establishment

Many of the species that are of potential interest for the farmers for planting have become rare in the natural forest and are only to an extremely limited extent available on the market (Lillesø *et al*. 2001a). The limited distribution of species and their non-availability on the market appear to have created a situation where farmers in a given area can only select among a relatively small number of the species that have potential for that area. Because farmers in different areas use different species, the total number of species used by farmers is large. Domestication strategies should therefore include development of seed sources for a correspondingly large number of species with different degrees of intensity.

The farmers use the large number of species for different purposes, and at different time of the year. There is no straightforward way of selecting high priority species for farmers' domestication in Nepal. A combined approach for developing initial priorities can include (1) identification of species suitable for specific conditions and providing valuable products ('asking the scientists'), (2) preference studies ('asking the farmers'), (3) market surveys and letting the markets determine which species that have a potential demand ('asking the market'). In the longer run, introduction of new seed sources and exchange of information between farmers may be a valuable tool for identification of farmers' preferences. It will in the end be up to the market to decide whether these species in the long run will attract sufficiently interest from the farmers.

In Nepal, the initial selection of species for domestication was based on the two first tools, 'asking the scientists', and 'asking the farmers'. This lead to initiation of so-called BSO programmes for the following 7 species: *Dalbergia sissoo, Bauhinia purpurea*, *Bauhinia variegata, Choerospondias axillaris, Michelia champaca, Bassia butyraca,* and *Phyllanthus emblica.* These species were mainly selected due to the small holders' recognition of their high value as timber, fodder and/or fruit trees. It is the intention that this species list should increase over time.

In addition, a number of species were targeted by establishment of so-called Farmland Seed Sources (cf. section 4 below), mainly in cooperation with two private seed enterprises that buy the seed from the respective tree owners (Dhakal *et al* 2005a) These species were: *Artocarpus lakoocha, Bauhinia purpurea, Bauhinia variegata, Boehmeria rugulosa, Choerospondias axillaris, Ficus auriculata, Ficus glaberrima, Ficus glomerata, Ficus hispida, Ficus lacor, Ficus neriifolia, Ficus semicordata, Ficus subincisa, Grewia optiva, Litsea cubeba, Litsea monopetala, Saurauia napaulensis, Azadirachta indica, Cryptomeria japonica, Eucalyptus camaldulensis, Flemingia macrophylla, Melia azedarach, Morus alba, Pinus patula, Tectona grandis*.

A further number of seed sources were identified in forests belonging to different Forest User Groups (FUGs). These covered the following species (Dhakal *et al* 2005a): *Acacia catechu, Albizia lebbeck, Albizia procera, Alnus nepalensis, Bombax ceiba, Cinnamomum tamala, Dalbergia latifolia, Fraxinus floribunda, Garuga pinnata, Michelia champaca, Phyllanthus emblica, Pinus roxburghii, Pinus wallichiana, Prunus cerasoides, Taxus baccata, Pterocarpus marsupium, Toona ciliata*.

4 Supplying tree seed to meet the immediate demand

Urgent seed insufficiency can easily lead to a perception, that the main challenge is to provide sufficient quantities of seed of the desired species in a sufficiently decentralised ways to make the seedlings easy accessible to the targeted farmers. That is a 100% focus on providing seedlings of the right species into the hands of the farmers. Under such conditions, genetic quality of the seed is rarely taken into account. Seed procurement is normally seen as an important activity that can be handled independently from the question of good genetic quality. The potential value of tree improvement and gene conservation may be recognised, but being a task for specific tree improvement and gene conservation activities – not something that should be the concern of tree seed providers. Such a perception of seed procurement is understandable given that many NGOs, governmental organisations and tree plantings projects face severe deficits of seed from species that can improve the livelihood for millions of farmers. However, the problem with the above ‘first seed procurement-then-genetic-improvement’ approach is that seed procurement in most cases will have substantial and direct influence on the genetic quality, whether purposely or not. This is because seed procurement involves a number of activities that has the potential to trigger strong genetic processes such as genetic bottlenecks (that can cause inbreeding depression and random genetic drift in present or later plantings), movement between ecologically different environments (that can lead to poorly adapted trees and initiate strong selection processes), or collecting seed from trees with unusual phenotypes (that can lead to over representation of specific traits in the distributed seedlings). If approached positively, these genetic processes may actually be used as an almost free engine for improved genetic quality and productivity. If ignored, they may decrease the productivity of the trees and thereby provide the farmers with suboptimal trees.

TISC have tried to accommodate the genetic factors by developing a strategy for immediate seed supply based on identification of two types of ‘above-average’ seed sources suitable for immediate seed collection. These two types are (i) Farmland Seed Sources (trees scattered in the farmland), and (ii) FUG seed sources (trees in forest managed by Forest User Groups). The basic idea is to reduce risk of mal-adapted trees by working within planting zones, reduce the risk of negative genetic selection by collection seed only of trees that are of above average performance, and reduce the risk of genetic bottlenecks by including seed collected from a fair number of un-related trees in a given seed-lot.

The issue of 'relatedness' between seed trees is complicated as many tree species in Nepal have been planted by the farmer from cuttings/seeds/wildlings from an unknown (but most often small) number of mother trees. In some cases the farmer can provide information on the origin, but it may be difficult to infer on the genetic basis of specific trees, and information often lack altogether. There is no simple and quick solution to the risk of high relatedness among trees on farmland. When establishing new improved seed sources, the problem can be handled through the design and breeding strategy, but for the trees that must serve for immediate production one must accept that the seed may be somewhat inbred as trees may be pollinated by close relatives. The problem with 'risk of relatedness' of the trees in the farmland can be partly addressed by including trees from a number of different farms. The idea behind this approach is that seed trees on different farms are likely to be less related than seed trees on the same farm, because farmers often have propagated trees by grafting (or seed collection) from older trees on their own or neighbouring farms. Collection of seed from a number of different farms therefore reduces the risk of procuring seed with very low level of genetic diversity. It will not reduce the average level of inbreeding in a given seed lot 'here and now', but it will increase the level of diversity and thereby reduce the risks involved in using trees over a large area that represents a genetically narrow base. Also, tree planting farmers may use their new trees for future seed collection. At that time they will benefit from being able to collect non-inbred seed because they planted trees from a genetically diverse seed source.

In order to be classified as *selected* seed source, the farmland seed source should meet a number of general criteria. These include (i) Basic documentation, (ii) Minimum number of healthy and vigorous trees have been identified, (iii) Minimum requirements on the distribution of these seed collection trees - in order to ensure that seed are collected on trees that have other trees of the same species growing within pollination distance (reduce risk of collecting selfed seed, see Table 1, below), (iv) all seed trees must be 'good trees' as evaluated by local farmers using the species (reducing the risk of negative genetic selection), (v) in case of unknown origin: seed trees should be distributed on several farms, depending on normal propagation method (see Table 1, below).

5 Improved seed sources through recurrent selection: The Breeding Seed Orchard concept

The tree improvement programme in Nepal was from the beginning in 1994 planned to build on the principles of the so-called BSO (Breeding Seed Orchard) concept developed by Barnes and co-workers in Africa (Barnes, 1984;1995). Due to the heterogeneous environments in Nepal, Domestication is intended to be implemented in a multiple population approach (cf. Namkoong *et al* 1980). A BSO is a seedling seed orchard that serves multiple purposes: (i) genetic test comparing trees within and between progenies, (ii) seed orchard for collection of improved seed, (iii) breeding population for selection of next generation plus trees. The BSO must be designed accordingly in order to accommodate all functions (Barnes, 1995; Dhakal *et al* 2005a). The approach has been identified as potentially suitable for many programmes in developing counties (Graudal & Kjær, 2001), and is especially appealing to the Nepalese context as it is based on low input, combines substantial gains with relative high level of genetic diversity in the plantings, and fits well into a decentralised delivery system (Kjær *et al.* in progress) . This expectation is supported by the results so far from Nepal, which suggest that the approach will lead to substantial genetic improvement for both *Dalbergia sissoo* (main product: timber) and *Bauhinia purpurea* (main product: fodder for cattle) (Jha *et al* 2006; Kjær *et al.* in progress).

Table 1: Recommended sizes for a classified farmland seed sources in Nepal according to TISC standards

SOURCE: (Slightly modified after Dhakal et al. 2005) Species characteristics	Minimum number of selected, superior trees	Minimum number of trees that must have contributed to a given seedlot	Minimum number of farms involved in collection to a given seedlot
Trees that farmers normally propagated by cuttings or grafting (e.g. Ficus)	50	30	15
Trees that farmers propagated from either seed or cuttings/grafting	50	25	10
Trees that farmers normally propagated from seed from multiple sources	50	20	5
Predominant pollinator	Maximum distance from the seed trees to other trees in the farmland seed source		
Beetles	100 meters		
Insects	200 meters		
Wind	200 meters		
Bats, birds, large bees	200 meters		
Fig wasps	1000 meters		

Note: Numbers are selected in order to obtain acceptable N_e given a number of assumptions

A number of steps are involved in the Nepalese seed source programme: (i) Survey gene pool for target species based on field observation within defined ecological zones, (ii) Selection of trees in the landscape and seed(/graft) collection, (iii) Establishment and maintenance of BSOs at one or multiple sites, (iv) Assessment and analysis of BSOs, (v) Genetic thinnings in the BSO leaving superior trees, (vi) Distribution of the improved seed. Each of the steps must be adapted to the specific species. The Nepalese programme operates with two levels of BSOs: intermediate level – where progenies are kept separate in the BSO, and simple level – where progenies are mixed prior to planting. In simple level BSO, genetic progress relay on simple phenotypic selection for 'ideotypes' (=best tree) in the BSO. However, as family structure is maintained in the intermediate level BSOs, these can be assessed for multiple characters; genetic parameters can be estimated, and combined within and between family selections performed. The assessment and estimation step can easily become a bottleneck in the programme if all BSOs are to be assessed, genetic analysis performed and result implemented for genetic thinning. Therefore, it is anticipated that most of the selections in the BSOs will be based on phenotypic selection 'within progenies' as this will be a simple operation (=select best tree within each plot). Such a strategy can simplify the selection procedure dramatically, and the loss in potential gain compared to a combined within and between selection regime will be small as long as long as heritabilities are not very low (Kjær *et al* in progress). Still, it is valuable to maintain family structure in at least some of the BSO for a given species, because it allows inference on genetic parameters of importance for guiding effective selection. It is *e.g.* valuable to be able to predict expected response (and correlated response) following selection for a given set of traits. Also, unforeseen events can happen. One of the intermediate level BSOs of *Dalbergia sissoo* (located at Sunsari, Eastern part of Nepal) has e.g. suffered from a severe attach of *Aristobia horridula* beetles killing a large fraction of the trees. Based on observation in the BSO, Dhakal *et al.* (2005b) could identify a clear genetic pattern with large differences between the Sissoo progenies in their degree of infestation. On the other hand, simple level BSO offers them selves to recurrent selection in decentralised BSOs spread over the country.

Will it work?

The experience from the Nepalese low input breeding programme has so-far been that technical shortcomings and biological obstacles do exists, but these can be addressed and handled without major problems. However, two key challenges remain to be addressed:

- Will the seed get into the hands of the farmers?
- Can financial sustainability and cash flow be maintained ensuring the programme to develop according to the plans?

The overall success of the programme is likely to depend more on these two issues that on the technicalities related to the development of the low input approaches. However, compared to other types of tree improvement programmes, we see the Nepalese low input programme as a god point of departure for obtaining good delivery and sustainability as well as good gains.

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Norway spruce breeding in Sazava river region, Czech Republic

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Abstract

Genetic experiment with half-sib progenies of 146 plus trees of Norway spruce (*Picea abies* /L./ Karst.) was planted on three sites in the central part of the Czech Republic in the late 70's. Initial assessment was performed at the age 15. Genetic parameters were estimated using ASReml statistical package and individual breeding values were predicted through the combined BLUP analysis for both plus trees and individual progenies. Currently, three students are measuring a number of quantitative and qualitative traits in all three sites, the updated assessment will then become their examination work.

The poster is focused on (1) the rough prediction of genetic gain available in the first cycle, (2) an attempt is made to declare three alternative breeding programs that differ in overall program investment. In the context of a rather conservative forestry in central Europe, we believe that a low-cost program with simple features will be the winner. Following this year's evaluation, selection will be carried on to initiate the long-term breeding program. Current goal is to speed up the delivery of genetic gain into newly-established forest plantations.

Keywords: Norway spruce, low-cost breeding, selection

Ex-Situ Conservation and Domestication Effects: Lessons Learned from Alpine Forest Tree Species

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Abstract

Artificial regeneration is one of the corner stones in the management programs for the conservation of endangered plant species. But "man made" regeneration is suspected to alter the gene pool of the progenies. We took two coniferous tree species for studying the domestication effects during nursery processing: a common species (*Larix decidua*) and a rare species (*Pinus cembra*).

Ordinary nursery stock was used and samples were taken before and after the grading and culling processes, respectively. Sample size was 120 plants for each of the analyzed provenances and grading alternatives. Metric traits (shoot length, root collar) were measured and bud samples were taken for isozyme analysis. Altogether, 14 enzyme systems (more than 20 gene loci) were analyzed and genetic diversity was expressed as gene diversity n_e, expected heterozygosity H_e, observed heterozygosity H_o and hypothetical gametic multilocus diversity Vgam.

The results verified the hypothesis, that nursery processing (culling and grading) provokes domestication effects but were not consistent in all cases. Further analysis of the data revealed different impacts on the gene pool which are caused by nursery processing. Consequences for the ex-situ conservation of tree species are discussed.

Keywords: *Larix decidua, Pinus cembra*, artificial regeneration, nursery processing, genetic erosion.

Seed quality of hybrid swarm populations Pinus mugo × P. sylvestris in Slovakia

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Abstract

Preliminary results of study on seed germinability in three putative hybrid swarm populations of *Pinus mugo* × *P. sylvestris* in Slovakia are presented. There exist some indications pointing to the reduced fertility of the putative hybrids compared to parental species but convincing evidence for this conclusion is still missing. The matter needs to be further analyzed.

Keywords: *Pinus mugo* × *P. sylvestris*, putative hybrids, seed germination

Introduction

There are several places in Europe where hybrid swarm populations of *P. mugo* × *P. sylvestris* have been reported to occur. In particular it is true of Rilla Planina and Rodopy in Bulgaria (Dobrinov 1965, Dobrinov & Jagdzidis 1971), the Nowotarska valley in Poland (Staszkiewicz & Tyszkiewicz 1969, Bobowicz et al. 2000) and Swiss Alps (Net-Sarquerda et al. 1988). In Slovakia such populations may be found in northern part of the country, on the localities Tisovnica, Habovka and Suchá Hora of the Orava region. Morphologically, the putative hybrid populations were characterized in terms of needle morphology and anatomy, cone size and shape and stem bark colour. Biochemically, serological characteristics of some individuals from a putative hybrid population *P. sylvestris* × *P. mugo* along with isozyme data have been provided (Prus-Glowacki & Szweykowski 1980, 1983). With special reference to Slovakian populations, Musil (1977) studied needle variation in hybrid complex *P. sylvestris* × *P. mugo* from Habovka. Viewegh (1981) has extended this study also of needle anatomy using cross-sections. Except for morphological variation of seeds analyzed by Musil (1977a), no data concerning fertility of the putative hybris swarm populations *P. mugo* × *P. sylvestris* have been provided. Presented study refers to the germinating potential of seeds from all the three putative hybrid populations in Slovakia.

Material nad methods

The hybrid swarm populations of *P. mugo* × *P. sylvestris* from localities in Tisovnica, Habovka and Suchá Hora were subjected to the study on seed germination along with the neighbour

population of *P. sylvestris* from Hruštín and with the two populations of *P. mugo* from the neighbour localities of the High Tatras (Štrbské Pleso, Roháče) and with one population of the species from the Low Tatras mountain ridge (Jasná). The list of populations studied is given in Table 1. Cones were collected from individual trees in 2004 and 2005. Their number ranged between 23 individuals of *P. sylvestris* from Hruštín and 49 individuals of putative hybrids from locality Suchá Hora. Because of small amount of seeds available in some trees, it was not possible to perform germination test in a triplicate. Therefore, only total number of tested seeds of a given population is taken into account in Table 1. The germination of seeds was carried out at 25ºC during the period of 21 days. Following statistical treatment, the average values of germination percentage were arranged as homogeneous groups (Šmelko & Wolf 1977).
A small number of mature seeds originating from putative hybrids were processed cytologically to illustrate histological structure of their embryos. Following removal of seed coat the seeds were fixed in Navashin fixative (12-14 hrs), washed under tap water, dehydrated in ethanol of increasing concentration, including its intermedium with toluene, and finally embeded into paraffine. After removal of paraffine from longitudinal sections of the seeds, the samples were stained in haematoxyline and embeded into canadian balsam. The permanent preparates were examined microscopically.

Results and discussion

The results of germination test presented in Table 1 give an impression of lowered seed germination in the putative hybrid populations.

Table 1. Seed quality in individual populations determined by in vitro germination test

Species	Population	Year	Number of tested		Germination percentage ± Std. dev.	Homogen. groups				
			Trees	Seeds						
P. sylvestris	Hruštín	2005	23	2607	52,4 ± 15,1			x		
P. mugo	Š. Pleso	2004	30	1500	93,5 ± 6,3					x
P. mugo	Roháče	2004	34	1077	0,5 ± 1,6	x				
P. mugo	Roháče	2005	30	1492	20,1 ± 19,4			x		
P. mugo	Jasná	2005	42	3609	3,0 ± 5,6	x				
Putative hybrids	Tisovnica	2004	45	3567	4,8 ± 6,0	x				
Putative hybrids	Habovka	2004	41	2692	8,6 ± 11,3	x	x			
Putative hybrids	Habovka	2005	30	673	23,4 ± 4,8			x		
Putative hybrids	Suchá Hora	2004	49	2408	18,1 ± 17,3			x		
Putative hybrids	Suchá Hora	2005	41	5275	50,9 ± 19,1				x	

Except for the population Suchá Hora producing 50.9% of viable seeds in 2005, the rest of tests with putative hybrids revealed much lower proportions of germinating seeds ranging between 4.8% and 23.4%. This figure contrasted with incomparably higher germinability of seeds in the populations of pure species *P. sylvestris* in Hruštín and *P. mugo* in Štrbské Pleso reaching 52.4% and 93.5%, respectively. However, uncertainty arises with respect to the control population *P. mugo* from the neighbour locality Roháče which in 2004 produced only 0.5% of germinating seeds. The population was comparable in this respect with the putative hybrid populations in Tisovnica and Habovka. The same was true of *P. mugo* population in Jasná which produced 3% of germinating seeds.

Table 2. Variance analysis of seed germination percentage based on 10 tests

Source of variation	Degrees of freedom	Sum of squares	Mean Square	F-ratio	Pr>F
Between groups	9	258578.07	28730.89	164.13	0.000
Within groups	340	59514.81	175.04		
Corrected total	349	318092.88			

Annual variation in quality of seeds registered in 2004 and 2005 in population *P. mugo* from Roháče and in hybrid populations in Habovka and Suchá Hora does not allow us to draw conclusion to whart degree are these differences conditioned genetically. According to Stern & Roche (1974) the low quality of seed at high elevations of mountains is due to either a low quality of functional pollen or due to pure seed maturity. In both cases the effect of unfavourable climatic conditions is postulated. This may be taken as an explanation of reduced seed germination in *P. mugo* populations from Roháče and Jasná. As far as the hybrid populations are concerned, it is reasonable to suppose that meiotic disturbances and abortive embryogenesis are due to the hybrid nature of their individuals. Reduced fertility of interspecific hybrids or even their complete sterility is generally acknowledged (Johnson & Kliman 2002, Flegr 2005).

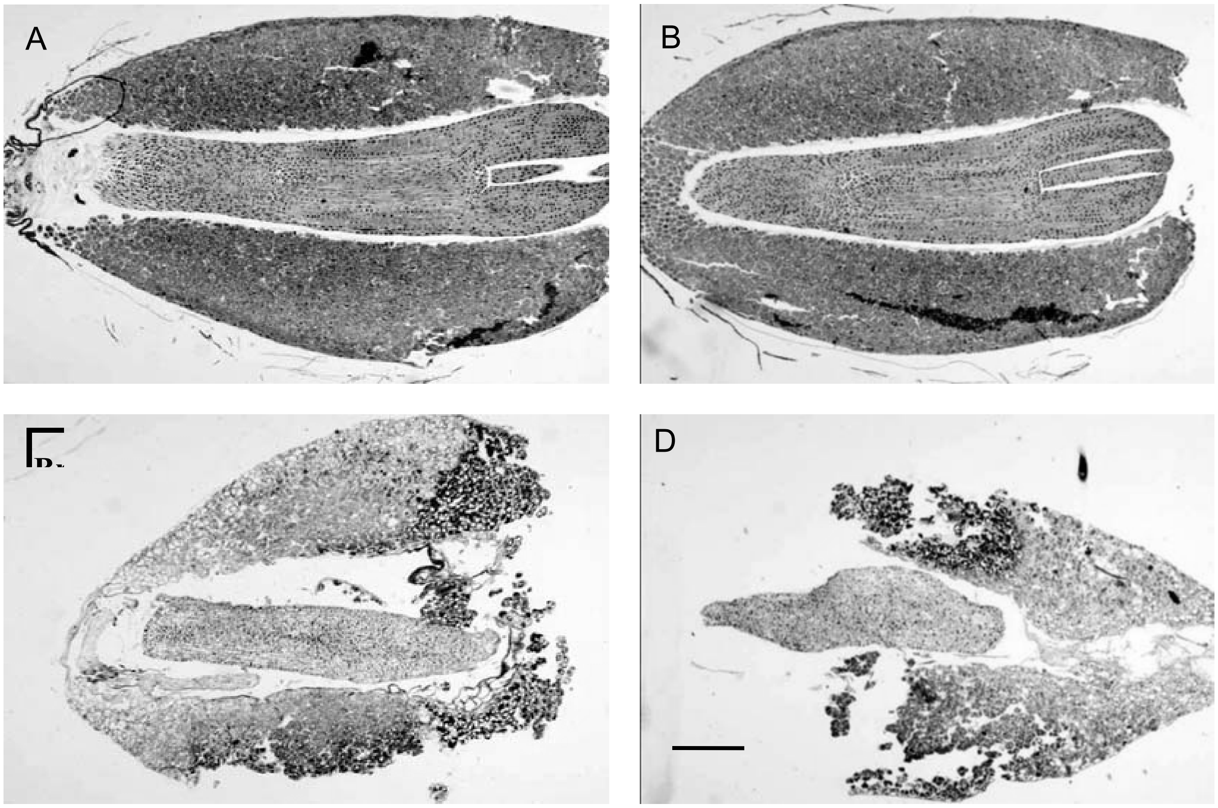

Fig. 1. Fully developed mature embryos (A, B) and underdeveloped embryos of the putative hybrids lacking cotyledons (C, D); bar indicate 500 µm

Our data indicate considerable reduction of pollen viability in hybrid swarm populations of *P. mugo* × *P. sylvestris* compared to the pollen quality in parental species (unpublished results) Also, at the level of mature seeds we have observed increased frequency of underdeveloped seeds in putative hybrids, prevailingly embryos without cotyledons. In some cases even earlier stage of embryogeny were affected resulting in a blokade of further development of young embryos in a corrosion cavity (Fig. 1). Nekrasova (1970) has ascribed this phenomenon in *P. sibirica* to the insufficient temperature sum during short growing season of the Altai mountains. Simak (1973) is of the opinion that abortive embryogenesis in *P. sylvestris* has also a genetical background. With special reference to the putative hybrid populations of *P. mugo* × *P. sylvestris* further experiments are necessary based on more extensive collections of seed material to shed more light on the problem of their fertility.

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Low Input Domestication of Tropical Trees

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Abstract

Current pressure to conserve the existing natural forest for conservation and other amenities is now giving prominence to the establishment of forest plantations to fulfill the need of the forest industries. Recently, the Government in its 9th Malaysian Plan has given emphasis to the development of planted forest for timber needs. In line with this priority a number of species have been recommended for planting. These would include among others Hevea, Hybrid Acacia and Teak.

To address the immediate need for good uniform planting materials, we have adopted the following short term strategy as part of a long term breeding plan to release some materials for use in the plantation programs. While, we have a dedicated Institute for the improvement and release of Hevea clones, our Institute, the Forest Research Institute of Malaysia (FRIM), has been tasked to provide improved teak and acacia hybrid planting material for immediate use for the plantation industry. This paper will discuss the work we have done to date in using mass selection as criteria to select parent plus trees with desired phenotypic characters and in using tissue culture to mass produce the plantlets required. For Teak we have done the selection from existing plantations and small holdings while for acacia hybrids we have selected from natural hybrids of crosses between A. mangium and A. auriculiformis from around the country. While the Teak is earmarked for the furniture industry, the Hybrid Acacia will be used in the pulp and paper industry. The growing cycle for teak is expected to be between 20-25 years while that of Hybrid Acacia will be between 6-7 years. In the next subsequent planting cycles we hope to provide further improved materials of these two species.

Key words: Tectona grandis, Hybrid Acacia, mass selection, plus trees, micropropagation

Introduction

For decades the natural forest has contributed significantly to the socio-economic development of Malaysia. To date timber and timber products stand supreme in terms of export earnings. During 2005, the exports were worth US$7.5 billion or 6.8% of the total commodity export. The export earnings are continuing to grow and this is mainly due to the rapid development of the wood based industry. This is the scenario we see presently.

The Malaysian Government also recognizes that forest conservation and the protection of the environment are important and have given emphasis to and highlighted these issues through various declarations and acts (Environmental Protection Act, 1985; Impact Assessment Order, 1987; Langkawi Declaration for the Protection of the Environment, 1989 and of the Forest Principles, 1994). The RIO Summit in 1992 culminated in a global recognition of the importance of the forest and the environment.

Moving back a little in time, it can be seen that Malaysia has a long and enviable track record for the management of their natural forests. Despite the rich natural forest present in the country, it has always been felt that a long term management plan was necessary to ensure their sustainable use. Plans with this in mind were developed with various activities such as enrichment planting, reforestation and afforestation schemes to be incorporated into the silviculture practices to ensure that logged over forests always remained in an economically productive state.

To demonstrate that Malaysia is committed to protect its natural forests, in the 8th and 9th Malaysian Plan (2000-2010) special emphasis has been placed to rehabilitate those badly degraded, logged over, encroached forests idle and ex-mining lands with forest plantation species. This would raise the value of the regenerating forests. The additional timber expected from these plantations would allow for the reduction in pressure on the other natural forest areas which could then be left aside for biodiversity conservation.

Brief History of Forest Plantations in Malaysia

Forest plantation is not a new concept and practice for Malaysia. However, interest in forest plantations lacked the required impetus in the distant past. It has vacillated quite a bit, influenced on one side from fear of impending timber deficits in the future and poor results from planting trials. Up until the 1970s, Malaysia was endowed with vast areas of natural forest. Under such superfluity, it was considered unnecessary and unnatural to convert natural forest into unstable monocultures. Like with the management of natural forests, plantation trials were first begun in Peninsular Malaysia, and then subsequently in Sabah and Sarawak. Record of planting forest species date back as far as 1880, when concern for loss of desired species was expressed (Hill 1900). Only in the last 10-15 years serious efforts have been placed to identify suitable species with superior qualities for short rotation forestry.

Species Identified for the Forest Plantation Program in Malaysia

According to regions, tree species have been identified for the plantation forestry program. The species have been classified as main and additional species (Table1). In all the regions emphasis has been placed on *Hevea* and *Acacia.*

Table 1: List of species identified for the Plantation Program

Region	Main Species	Additional Species
Peninsular Malaysia	*Acacia* hybrid *Hevea brasiliensis*	*Tectona grandis* *Khaya ivorensis* *Azadhrichta excelsa*
Sabah	*Acacia* hybrid *Hevea brasiliensis*	*Tectona grandis* *Octomeles sumatrana* *Neolamarkia cadamb*a
Sarawak	*Acacia* hybrid *Hevea brasiliensis*	*Neolamarkia cadamba* *Paraserianthes falcataria* *Octomeles sumatrana*

Breeding programs for Short Rotation Forest Tree Species in Malaysia

About the 1900, rubber production became the new excitement. In 1901, the first 180 acres of regular plantations of Para rubber (*Hevea brasiliensis*) was planted in Peninsular Malaysia. For almost 100 years Hevea has been planted in Malaysia for latex and now over the last 20 years its timber is highly sought after for the furniture industry. Extensive research on Hevea has been carried out by the Rubber Research Institute Malaysia now know as the Malaysian Rubber Board and they now have excellent clones both for latex and timber for their numerous breeding trials carried out over the years.

For the other selected timber species, tree improvement trials were started in the mid 19th century, mainly on an *ad-hoc* basis. Serious improvement programs (involving a sustained concerted effort to improve the genetic stock) started only in the last 10 years. Hence for many of these selected species we do not really have improved seeds or clones except for some clones of teak and acacia hybrid that we had started evaluation in the early years.

There is now a direct order from the Government to plant close to 375, 000 hectares as forest plantations in the next five years and there is now a serious pressure to come out with good planting stock that can be made available to nurseries and companies that are carrying out the planting programs.

Table 2: Timber Latex Clones released for the Forest Plantation Program

Clone	**Latex Yield (kg/ha/yr)**	**Clear Bole Volume (m3/tree)**	**Canopy Wood Volume (m3/tree**	**Total Wood Volume (m3/tree)**
RRIM 929	3,148	0.60	0.60	1.20
RRIM 2001	2,850	0.41	0.82	1.23
RRIM 2002	2,348	0.44	0.66	1.10
RRIM 2008	2,686	0.33	0.99	1.32
RRIM 2009	2,277	0.34	0.34	0.68
RRIM 2015	2,760	0.43	0.87	1.30
RRIM 2016	2,582	0.43	0.85	1.28
RRIM 2020	2,232	0.36	0.64	1.00
RRIM 2023	2,822	0.35	0.46	0.81
RRIM 2024	2,685	0.52	0.74	1.26
RRIM 2025	2,700	0.63	1.24	1.87
RRIM 2026	2,204	0.66	0.45	1.11
RRIM 2027	3,036	0.60	0.70	1.30
PB 260	1,633	0.37	0.93	1.29
PB 355	1,397	0.53	1.06	1.59

(Source: MRB, 2003)

Strategy adopted by FRIM to make available improved planting material for the immediate needs.

We report below the work that has been done for *Hevea brasiliensis*, *Acacia* hybrid and *Tectona grandis* for which we have planting materials that have been evaluated, *albeit* not all clones have been vigorously tested to ensure their versatility on a wide range of soils types particularly for *Acacia* hybrid and *Tectona.*

Hevea brasiliensis

For Hevea, extensive work had already been done by the Malaysian Rubber Board with regards to breeding. In the early past, focus was only on latex yield but over the last 15 years they started to breed for both latex and timber yield. Recently, they have released a list of 15 clones that could serve a dual purpose for both latex and timber yield. The proposed clones with details on yield are given in Table 2:

Large scale production of these clones is carried out by bud grafting of the desired clones on compatible root stocks raised from seeds. This procedure is well established and planting material in large quantities is raised in this manner.

Acacia hybrid

Currently we have around 40 ortet selected from various locations all over the country where the natural acacia hybrid occur. This hybrid is a cross between *Acacia mangium* and *Acacia auriculiformis* and they have characteristics that are far superior than their parents in relation to susceptibility to heart rot and bole straightness. The selection criteria used is in relation to height, stem diameter, volume, straightness, branch frequency and angle, branch diameter and health. Currently 5 of these clones have been tested extensively and have been found to be suitable in multi location trials. The other clones are currently being bulked up for trial. In addition we have now initiated a control crossing program to develop clones with desirable wood density and fire length. Details of the crosses that have been carried out and number of selected clones for trials are outlined in Table 3

Table 3: Controlled Breeding Program in Acacia

Details	Quantity
Controlled crosses done	70,000
Seed pods formed	380
Seeds harvested	2,000
Seedlings produced in vitro	1,400
Selected clones	400
Plantlets for clonal trial	40,000 (100x400)

Tectona grandis (Teak)

Teak is also not a native species but was introduced into the country a few hundred years ago. Today this species has become a landrace and has adapted itself to some regions of the country. Particularly in the northern region of the country, where the climate is somewhat pronounced monsoonal in nature, this species has adapted well there. We have identified close to 50 ortets based on phenotypic characteristics similar to the criteria used for selecting the acacia ortets. These parents have been used to initiate the cultures (Table 4). We have field data on about 10 of these clones and of these we have recommended 4 clones initially for forest plantation establishment in the new program.

Table 4: Details on the *Tectona* Clonal Program

Details	Quantity
No of ortets identified	50
No already tested	15
No. selected for release	5
Number awaiting trials	35

Advantages of Clonal Forestry

From the breeder's perspective, clonal forestry has the advantage of being able to make additional gains, which may be obtained by capturing non-additive genetic variation. It allows the capture and transfer of all of the genetic potential from the donor tree to its vegetatively propagated offspring. In this manner high gains can be made if the best trees are propagated asexually when compared the selection of seeds from the best individuals. The other advantages as highlighted by Eldridge et al (1994) are uniformity, adaptation, cost, wood production, deployment of GMOs, deployment of hybrids and expensive rare seeds and also the opportunity to gain a better understanding of individuals over time and over sites.

Disadvantages of Clonal Forestry

The most important hazard especially in using monoculture or only a restricted number of clones is the risk of heavy losses due to insect or fungi attacks on clones that are highly susceptible. The risk obviously is greater when large areas are planted and when long rotations are used. The risk is normally overcome by planting a relatively large number of clones. Common and best practice is to manage the risk by limiting the number of clones in production by limiting the total area that may be planted with a single clone.

The aging of material can be another problem in the propagation of clones. While micropropagation is considered as a method to rejuvenate older trees, however, works on some species suggest that although micropropagation has a rejuvenating effect, the level of juvenility is not the same as that of the starting material and that in vitro induced juvenility was rapidly lost with time. If this happens, it could be an important stumbling block when testing clones. Clones have to be tested in long term field tests before mass producing them. By the time the clones have been tested, the original mother plant material will have reached an age where propagation may become difficult. This can be overcome if part of the parent material is cryopreserved (Park *et.al*, 1998) to keep the juvenility or to initiate new cultures from the original desired parents.

Although gains from asexual propagation exceed the gains from sexual propagation, continuous genetic progress relies on a long term breeding program based on sexual reproduction. The breeding program has to ensure that there is continued supply of improved materials for desired traits.

Conclusion

For immediate use in our plantation programs, we have adopted a mass selection criterion to identify potential ortets based on desired phenotypic characteristics. These have been in-vitro propagated for field trials in multi-locations. Stable and well performing clones are then selected for release in the first generation planting. Subsequently we will be providing further improved planting material following our conventional breeding programs and rapid propagation systems that are in place.

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Genetic Variation in Four Natural Populations of Taurus Cedar (*Cedrus libani* A.Rich.)

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Abstract

Genetic variation in four natural populations of Taurus cedar (*Cedrus libani* A.Rich.) in Turkey was determined with the aid of isozyme analyses. Isozymes from 12 enzyme systems being extracted from haploid female gametophytes of the seeds were separated by horizontal starch gel electrophoresis. In 12 enzymes systems, 16 loci and 37 alleles were observed. The average proportion of polymorphic loci for the populations ranged from 43.8% to 62.5%. The average number of alleles per locus per population was 2.0. The mean expected-heterozygosity (He) of the populations was 0.165. The level of gene flow (N_em) was found to be 12.7 per generation. Rather high proportion of genetic variation was due to differences within populations (98.07%). Nei's genetic distance coefficient ranged from 0.003 to 0.008 among the all possible population pairs. The mean value of Nei's genetic distance (0.005) confirmed the hypothesis that the level of variation among the populations is low. The results suggest that populations of Taurus cedar might have relatively high adaptability to be planted on wide array of ecological conditions within its geographic range.

Keywords: Lebanon cedar, isozymes, geographical variation, starch gel electrophoresis

Low-Input Tree Breeding Strategies

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Abstract

Low-input strategies for seed production and breeding are discussed. Among such strategies are: phenotypic selection; inefficiency of progeny-testing; absence of testing; unidentified plants; combining objectives; genetic unbalances; seed collection areas; forwarding the breeding population by open pollination instead of controlled crosses; and for some cases vegetative propagation. Sustainable and optimally used low-input strategies require advanced quantitative genetic considerations, in particular concerning the management of relatedness.

Introduction

The development of tree breeding as a science is mainly driven by the needs identified by the large high-input programs. Consequently, methods suitable for high-input programs are too often implemented into low-input programs also. Sophisticated or futuristic tree improvement, which requires large resources, stable funding, efficient monitoring, and control, competent and permanent technical staff and well-developed infrastructure, is well represented in the qualified literature, in scientific research, in available expertise and in formal training programs. In contrast, methods particularly suitable for breeding in resource poor situations may be neglected or used inefficiently, as little thought is given to adequately develop implement and optimize these methods. There is rather little qualified scientific thought given to low-input breeding strategies, and not much is found about it in the more established literature. In the few more known examples of high-lighting low-input breeding (e.g. Namkoong et al. 1980; Shelbourne 1992; Barnes 1995), the focus is still on rather fancy techniques, and little effort is made on optimizing real low-input programs. Tree breeders generally feel more motivated to focus attention on how well things can be done rather than how cheaply (but somewhat sloppy) they can be done. There is no sharp delineation between low-input breeding and high-input breeding and any effort to focus on low-input strategies, as is the purpose of this paper, will unavoidable be hampered by this over-simplification. Low-input breeding can be characterized as cheap, convenient, simple, robust, survives periods of neglect, local, requires little record keeping and central control, limited need of monitoring and control, not dependent on high tech or specialized competence and is possible to run on a small and uncertain budget.

The aim of this lecture is to stimulate implementation of; research about; and development of low-input techniques in forest tree breeding. Similar stuff has been presented in lectures by Lindgren (2000 and 2003). Focus will be made on phenotypic selection, inefficiency of progeny-testing, absence of testing, unidentified plants, combining objectives, genetic unbalances and relatedness control, and forwarding the breeding population by wind pollination instead of controlled crosses.

Short time strategies

Good provenances in natural forests can be identified and seed collections can be guided by such provenance test information. Good stands in a setting similar to where the material is used can be identified and used for seed collection. Stands intended for seed collection can be phenotypically thinned to improve the pollen source. Seed collection can be guided to the best trees. Such techniques are always used in the early stage of forest tree breeding, in low-input situations no further actions may be planned, but in this lecture a situation where continuous improvement is intended is discussed. Probably it is recommendable to always have thoughts about long term strategies when a species is actually used for planting to some extent.

Combine populations!

The breeding stock can be divided into production population, breeding population, candidate population, multiplication population, archives, gene conservation population, mass-multiplication population, testing population. For low input breeding, it is essential to combine different objectives in the same physical groups of trees. Seed collection areas or seedling seed orchards can in the same time be productive forests, candidate population, testing populations and serve gene conservation purposes. Such combinations can be headed for at planting.

Open pollination or controlled cross?

In low-input breeding situations, one of the most important considerations is if controlled crosses are needed. Controlled crosses appear in most circumstances as complicated and demanding operations requiring special skills and subject to unpredictable outcome and mistakes. Grafting is often done and that includes large costs and long time delays. Pollen need to be harvested, extracted and stored. Open-pollination is cheap and simple. It offers the advantage that decision about seed collection can be done when it is known that sufficient amount of seeds can be collected. Open pollinations may increase the breeding efficiency much as generation turn over can be much faster. Open pollination has advantages for gene conservation as it draws genes from many ancestors and preserves diversity (Wei and Lindgren 1995). Open pollination could be applied so it yields higher gain than single-pair mating (Wei and Lindgren 1995) or other crossing designs (Wei et al. 2002).

There are two different advantages in using controlled pollinations in forwarding the generations of the breeding stock. The evident one is to control pedigree and relatedness, but another is to avoid inflow of unimproved, unknown, and variable pollen into the breeding stock. For wind-pollinated species which occupy a large share of the land, it is common that fertilizing pollen originates quite a distance from the mother. E.g. in seed orchards of major wind-pollinated species it is common that half of the fathers are to be found outside the orchard (e.g. Lindgren and Prescher 2005). In such situations the inflow of pollen will reduce the improvement effect considerable, and the open-pollination alternatives may appear inferior even in a low-input situation. It may be possible to isolate the breeding stock from other pollen sources by distance or in other ways, e.g. in a plastic green house or isolation by differences in the phenology. The isolation of the breeding population may not need to be 100%, e.g. gene migration by 5% pollen inflow may be quite tolerable in a low input situation even if 50% inflow usually will be regarded as unacceptable much. If a wind-pollinated species is not dominating (the case for many exotics) it can be easy to find a location where physical isolation is sufficient. For non wind-pollinated species the behavior of the pollen vectors are quite important. Careful considerations about pollen inflow should be done before deciding to rely on open pollination for continued breeding.

A low input breeding strategy without controlled crosses can utilize the production population as candidates for the next breeding population. That is very cost-efficient. A consequence is that the breeding population is difficult to structure in unrelated lines, and thus in the long run inbreeding and inbreeding depression will occur in the production population. A common tool to create a production population is to create a seed orchard with selected individuals, in the considerations for what trees to place in a seed orchard, the occurrence of relatives is an important factor (Olsson et al 2001), but this can be considered only if there are pedigrees. Considerations about these factors have to be done. It is likely a low level of inbreeding in the production can be tolerated and is optimal compared to the consequences of eliminating it. If multi-function plantations of seedling seed orchard character are used for seed production, some degree of inbreeding is unavoidable. For inbreeding considerations, however, actual calculations are desirable. When low-input breeding is practiced, clonal seed orchards or breeding structured in sublines are seldom considered, thus these possible disadvantages with uncontrolled pedigrees are not important. A general remedy against inbreeding in future production populations is to start with a sufficiently large breeding population. It seems risky to initiate a long term program with less than a hundred open-pollinated families.

An emerging technology is to find out the pedigree of breeding stock by markers. It can be limited to fathers if maternal pedigree is controlled by design. In a more futuristic perspective more distant ancestors can be identified. Parental identification “afterwards” makes it possible to control coancestry, selfing, inbreeding and gene migration without proceeding over generations with controlled crosses. Breeding systems with retro perspective pedigree reconstruction goes well in hand with the low input techniques open pollination and phenotypic selection. Currently it is doubtful if this should be considered in low input breeding, but e.g. if the program heads for creating options for future expansion, when pedigree reconstruction may be an option to consider in the strategy when the input level increases, e.g. if a marginal species becomes more important.

Phenotypic (Mass) Selection has appealing characteristics for low input breeding
Phenotypic selection can be done fast and at a low cost. The generation turn over can be increased. The increase in breeding efficiency by these features is often important enough to compensate for draw-backs.

Phenotypic selection (mass selection) means selection based on the appearance of the individual tree itself, not considering the performance of its relatives. The phenotype of a tree (compared to other trees) gives an estimate of its breeding value (=phenotype*heritability). It may seem an unsophisticated philosophy to rely on the phenotypic appearance, but actually many improved seeds used in plant production today rely mainly on phenotypic selections. E.g. the Swedish plant production was 2005 60% seed orchard origins, but the concerned seed orchard selections are completely dominated by phenotypically selected untested plus trees. Although more advanced seed orchards have been established in Sweden, and some of them come into commercial seed production, their impact on plant production is still marginal. Also genetic thinning or selective cone harvest is uncommon. Thus, those in favor of low-input can be said to be in good company if relying on phenotypic selection. As forests derived from phenotypic selection of plus trees has existed for many decades, it can be claimed to be the method with which forest tree breeding and forestry has the longest experience, and this experience is generally a positive one. It can thus be argued to be a safe and method based on long experience.

Phenotypic selection is the method used by *Nature* and is the driving force for evolution, and has thus existed as long as Life. Life has evolved by phenotypic selection; it is the fundament Life rests on and the cause of Life. It offers comfort to use a "natural" method; even if it seems unsatisfying that modern developed *Science* has difficulties to do better than *Nature*, as discussed below. The similarity to *Nature* seems a strong argument to believe that it is basically sustainable, natural and environmental friendly. That a method is similar to *Nature* can be regarded as a powerful argument to use it. It is very simple and it seldom requires sophisticated training. The selection situation may be more similar to forestry practice (e.g. random mixture of genotypes planted as operational forestry, which will be more similar to the conditions selected for, than genotypes arranged in plots under experimental conditions). It minimizes or eliminates the need for experimental layout and documentation. It does not use a complicated calculation algorithm that often works like a “black box”, where severe mistakes easily may pass undetected. It is cheap. It is easy to keep track of data. In contrast to selections based on genotype testing much of the variation in the characters selected for will remain among the trees selected to constitute the parents of the following generation, at least if heritability is not extremely high. Testing increases the accuracy (it can be viewed as making heritability high), and will considerable reduce the variance in the characters selected for. Phenotypic selection has attractive properties for combining tree improvement and gene conservation in the same operation. This advantage may however be smaller than it may appear at first sight, as much of the variation comes back after recombination even after intensive accurate selection.

Phenotypic selection has effects on gene diversity as selected trees are likely to be more related than unselected. To study the magnitude of this increase in relatedness, a trial with 98 open-pollinated families of Scots pine was (hypothetically) thinned based on tree height (Fedorkov et al 2005). First when less than about 10% of the initial trees remained, notable effects on potential inbreeding, relatedness, status number and diversity was noted. To retain almost all families a somewhat more relaxed thinning leaving about 20% of the trees was needed. The study suggests that rather intense phenotypic thinning (leaving more than 15%) has the ability to raise the genetic gain some percent without many problems with accumulation of relatedness. This can be used as a tool in low input breeding. It seems a reasonable praxis to thin intended seed collection stands. For Scots pine in Sweden it is a rather frequent praxis to leave many trees as seed sources for natural regeneration. When doing this some could be headed for seed collection area, when special attention could be made to leave good trees, and some documentation done. When the trees finally are felled, cones could be collected. The thinning would also improve seed production, and as the stand density is lower there will be less trash on ground to search, thus such praxis has not just genetic advantages.

Phenotypic selection, without knowing or caring about the pedigree of the trees, has in several studies been found to be about as efficient for long-term breeding as if the pedigree was known and used as an aid in selection (e.g. Wei 1995; Andersson 1999). One of things a breeder loses by using phenotypic selection is the deliberate choice of emphases on gain versus gene diversity. It may be possible to mend that by adjusting the selections, e.g. by restrictions, but when it is not pure phenotypic and probably less efficient. Numerical simulations indicate that phenotypic selection often results in an intuitively appealing compromise and seldom results in a drastic increase in relatedness (e.g. Andersson 1999).

Selection based on the phenotype needs not depend on actual measurements, i.e. a subjective evaluation of all characters of a tree (including its performance compared to its neighbors) can be made in the field and need not be calculated and evaluated in the office at some later time more separated from the actual event. Such an evaluation may actually be more accurate than an "objective measure", as all characters can be jointly considered including the local environment (thus relating to the performance of the neighbors). A field evaluation and selection does not depend on a long chain of actions on different places and by different people where mistakes or delays may easily occur. In practical selection, there is usually a considerable amount of subjectivity at the final stage in a decision procedure; this cannot be circumvented by using high-input techniques in the office.

Phenotypic thinning for unpedigreed seed production areas and also plus tree selection have been discussed, advocated and applied e.g. by Harwood et al. (1996). As low-input breeding must usually rely on phenotypic selection, it must be pleasing for those applying it to know that it is now acknowledged by modern science as an efficient tool (see below). Thus, seed production areas can be established, and the resulting forests later creamed for the best unpedigreed trees, whose descendants are used for establishing new seed production areas, making low-input breeding sustainable over multiple generations, provided the tree numbers involved are sufficiently large.

In a study of the genetic gains obtainable from a range of alternatives, Shelbourne (1992) found that phenotypic selection in unpedigreed stands, although somewhat lower in genetic gain than more elaborate strategies, still seemed favorable when possibilities for faster generation turn-over and lower cost was taken into account.

Relatedness and Gain

Forest tree breeding can be said to aim for creating options to get regeneration material combining high genetic gain with desired diversity. Here we mainly focus on diversity as gene diversity loss since the improvement operation started. This gene diversity can be quantified as group coancestry or status number.

Relatedness has evident disadvantages and can not be neglected. Relatedness is the likelihood that genes interact, and that is proportional to the square of there frequency, thus the disadvantage of relatedness can be quantified by the square sum of genetic contributions. Gene diversity can be interpreted more or less equivalent to relatedness. It can be measured "absolute" by observations of alleles equal in state by expected average heterozygosity, which is a square sum of contributions of alleles. It can also be measured relative to a reference population ("loss of gene diversity") as square sum of the genetic contributions. This is the basis of concepts like status number, relative status number and group coancestry, which are measures built on average relatedness. Group coancestry can be interpreted as a measure of *the loss of gene diversity* since the initiation of tree breeding, thus one minus group coancestry. Gain is the sum of the gain of the genetic contributions; so much of the act of balancing is to consider a sum versus a square sum.

If where exists knowledge about how trees are related, more accurate estimates of breeding value of individual trees can be made which use the information from relatives. If a given number of trees are selected with such more accurate breeding value estimates as a criterion, the genetic gain will be higher. There is a best way of estimating breeding values utilizing the information about how trees are related, for more complex relatedness situations these techniques has developed into an art of its own.

By considering the performance of the relatives, a greater gain can be achieved at the same selection intensity, but the additional gain is accompanied by a correspondingly higher relatedness among the chosen individuals. Actually a philosophy to maximize gain may often result in inoptimal tree breeding programs.

Phenotypic Selection vs. combined index selection

In the simplest situation when where is a simple family structure, the best way of combining values for families and for individuals to get breeding value estimates and when select for them is usually called combined index selection. While selecting among the first generation of offspring, selected trees will be sibs to a higher extent, if sib performance is considered when constructing the selection criteria. In examples relevant for forest tree breeding this overrepresentation was shown to be drastic (Lindgren and Wei 1992) and other ways than combined index selection maximizing gain has to be searched for. The classical selection strategies are compared in Figure 1. The graph is generated by an optimizing selection procedure using the same selection intensity for all diversity values (Lindgren, Wei and Bondesson 1993). There is no way to get a higher gain given the diversity. “Linear deployment” (which is optimal for unrelated clones, Lindgren and Matheson 1986) from unrelated families are usually indistinguishable close to optimal (Wei and Lindgren 1995). Between family selection results in the lowest diversity and in the same time the gain is not at top, so that is an appealing strategy. Although combined index selection maximizes gain, it does so at a dear price in diversity. Within family selection maximizes diversity, but minimizes gain. Phenotypic selection appears as a good compromise between gain and diversity. In the way this curve was drawn phenotypic selection appears as an optimal selection procedure. The calculations assumed an infinite number of large unrelated full-sibs and considered only the first round of selections.

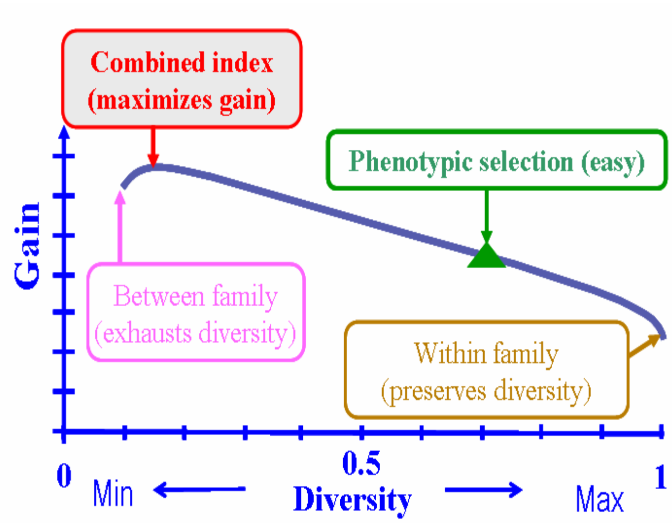

Figure 1. Relation between Gain and Diversity (Relative Status number) following selection in a population with a symmetric family structure. The graph is generated by an optimizing selection procedure. To choose the best families minimizes diversity. Maximal gain is obtained by combined index selection where family and phenotypic values are optimally weighted. But it results often in a very low diversity. Within family selection maximizes diversity, but minimizes gain. Phenotypic selection

appears as a good compromise between gain and diversity. The candidate population was composed of an infinite number of large unrelated full sib families of equal size. Heritability was 0.25 and 10 percent of the individuals were selected. Figure 1 is modified from Lindgren and Wei (1994).

More complex and more realistic situations can be dealt with by simulations. The outcome of breeding programs based on phenotypic versus combined index selection at the same genetic diversity, POPSIM© (developed by Tim Mullin) was used. In practice a sufficient diversity has usually been achieved by restrictions on the number of progeny per parent selected, and such restricted selection has been employed. Figure 2 is based on calculations and results presented in Andersson (1999) and Spanos et al. 1996. The simulated breeding program created a recruiting population by single pair mating and selected the best according to either phenotypic selection or combined index selection over a variety of restrictions. Balanced selection means that 2 individuals in each SPM family were selected (the outmost left in the graphs). The size of the breeding population was kept constant over generations and among compared alternatives.

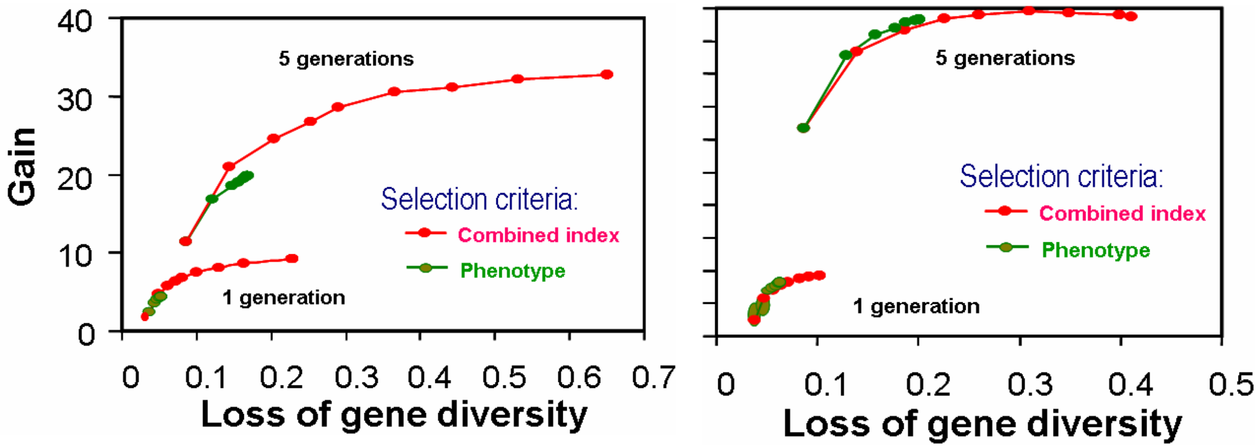

Figure 2. A comparison between selection for combined index (approximately the best estimate of breeding value) versus mass-selection (phenotype). Gain is shown as a function of the loss of gene diversity for simulations of a multigenerational tree breeding program with breeding population size 20 and single pair mating. The points on the graphs correspond to restrictions on the number of offspring selected from each parent. The leftmost point is for 2 selections which corresponds to within family selection or balances selection, where the two top individuals are selected with both criteria. The rightmost point is for unconstrained selection. The left figure is for heritability 0.05 and family size 500, the right figure for heritability 0.5 and family size 20. The figure is based on calculations by Andersson (1999) and others.

When compared at the same loss of gene diversity among the selections and the same selection intensity, phenotypic selection and combined-index selection produce approximately the same genetic gain (Figure 2 and Wei 1995). Phenotypic selection seems to be competitive for achieving genetic gain at a given level of gene diversity even in multi-generational programs generating complex and unbalanced pedigrees, see the upper graphs in Figure 2. Although phenotypic selection appears approximately as good as combined index selection, phenotypic selection is slightly superior when heritability is high and family size small (right part of Figure 2) and slightly inferior when heritability is low and family size large. Clonal testing can be seen as a way of increasing heritability and decreasing family size, thus clonal testing makes phenotypic selection (of the best tested clones) relatively more attractive. The family information gets generally more informative in situations with low heritability and large families, and thus combined index selection is more powerful in such situations (left part of Figure 2).

When only the initial offspring or the development a the first generations is considered, it may be argued that selection for breeding value (combined index selection) are able to produce a much higher genetic gain than phenotypic selection (Figure 1 and 2). Foresters may not care that this is associated with a large reduction in gene diversity; diversity may not be seen as a problem for the production forest. This can be considered a powerful argument against phenotypic selection in the short time perspective. Reduction in gene diversity will, however, lead to reduced gain in later generations, so after around five generations the *maximum* gain obtainable can for some situations (e.g. right part of Figure 2) be about as high for phenotypic selection as for combined-index selection (Andersson 1999). These findings concerned with forest tree breeding are supported by similar results by animal geneticists (e.g. Quinton et al. 1992). Exhaustion of the genetic variation leading to reduced gains by unconstrained selection, in particular if combined index selection or still more powerful estimates of breeding values can occur in a few generations (right part of Figure 2). Reliable breeding value estimates by progeny testing can increase this risk. Such exhaustion is higher in high input breeding and in that sense high input breeding can be seen as a higher risk than low input breeding, hopefully that disadvantage of high input breeding is compensated by a higher competence.

The main alternatives balanced selection, unrestricted phenotypic selection and unrestricted combined index selection (=breeding value estimate) are compared in Figure 3 in a way intended to facilitate comparisons focusing on the effect of early exploitation. It suggests phenotypic selection compared to balanced selection offers a faster accumulation of gain in a shorter time at the cost of a rather small sacrifice in accumulation of gene diversity, while combined index selection erodes the diversity too fast compared to the achievable gain even under conditions chosen to be favorable for combined index selection.

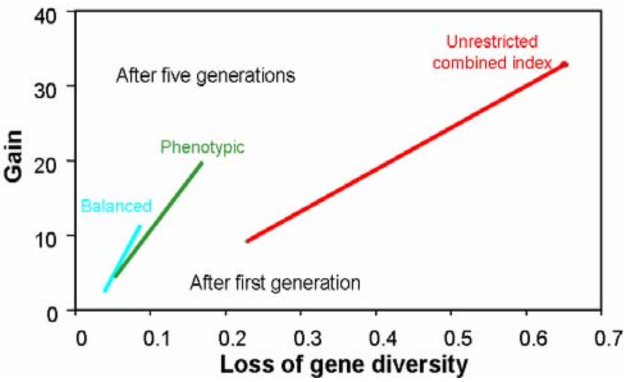

Figure 3. The gain as a function of loss of gene diversity for three strategies (balanced within family selection, phenotypic selection and combined index selection (both without restrictions) during a breeding program spanning over five generations. Heritability is 0.05 and family size 500. The graphs connect the situation after the first generation (leftmost) and five generations (rightmost). The figure is based on calculations by Andersson (1999) and others.

Breeding population size could be dealt with as a variable for fair comparisons

The comparisons in Figure 2 and 3 deal with selection strategies in breeding programs where several factors are dealt with as fixed. The comparisons kept breeding population constant among selection strategies. It can be said to be a common praxis among breeders to first fix the size of the breeding population before giving consideration to other factors in the breeding system. That does not make the basis for fair comparisons, the breeding population size should be considered as a variable the breeder can control and optimize joint with other inputs under the breeders control as done by Danusevicius and Lindgren (2005). Combined index selection tends to erode the diversity fast, but that tendency can be compensated by keeping a larger breeding population size, while balanced or phenotypic selection preserves more of the gene diversity and can thus focus resources on other parts of the breeding system than maintaining a large breeding population size. A comparison between phenotypic selection and combined index selection with breeding population size as a variable and the total number of plants as the fixed resource for the

breeding program was presented by Li and Lindgren (2006). When gain was compared at the same gene diversity under these conditions, the following was noted:
The alternatives became similar when gene diversity (status number) became large. “Sufficient” high diversity to make combined index selection an acceptable alternative seems to be achieved if the breeding population size is above 250. As generations pass phenotypic selection resulted in higher gain. At low heritability, combined index gave more gain.

However, an additional member of the breeding population means added cost and efforts, and this cost is very important for the optimal size of the breeding population (Lindgren et al 1997). Breeders also often feel constrained by earlier decisions about breeding population size. Even if the results by Li and Lindgren (2006) in a way explains why combined index selection appears doubtful in spite of that it can be considered theoretically optimal, it can not be interpreted as a powerful argument against phenotypic selection.

Testing

The simplest way of selecting good individuals is to rely on their phenotypes. Progeny testing or clone testing can be seen as a way of boosting the heritability. A relevant question if there is place for genotype testing in low-input programs. Testing requires field identities, recorded pedigrees, documentation, long-term planning, long-term co-ordination of activities, organizational stability, and a breeding budget which remains in decades and, often, clone archives. A strategy including testing means a large long-term investment, which seems possible to justify only in situations when it seems certain the results will be utilized and appreciated in the future.

The value of testing vs. phenotypic selection in conditions similar to the Swedish pine and spruce long term breeding was recently analyzed by Danusevičius and Lindgren (2002, 2006). It was concluded that clone testing was the best strategy, followed by phenotypic selection (Figure 4).

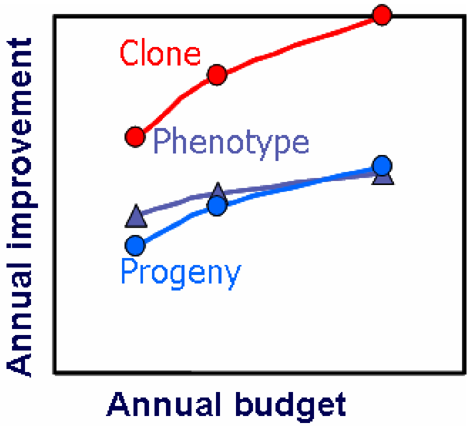

Figure 4. Comparison between different testing strategies as a function of the annual cost per parent. Clone testing was much superior at all cost levels. Phenotype testing was better than progeny testing if the budget was low. The inputs are chosen to be representative for Swedish conifer breeding. The figure is based on calculations by Danusevicius and Lindgren (2002).

It is more favorable to select genotypes for continued long term breeding based on their performance as clones than based on their unreplicated phenotypes or progeny. Clone testing is operative for Norway spruce and used routinely in breeding in Sweden. Phenotypic selection becomes more superior compared to progeny-testing the lower the budget and the higher the heritability. The optimal breeding cycle time with alternatives involving progeny-testing spans over several decades for long-rotation forestry. Thus progeny-testing seems an alternative worth considering only for breeding programs of economically very important crops with a stable long term breeding commitment.

A study by Ruotsalainen and Lindgren (1998) showed that, with few exceptions, if offspring was generated with pollen as good as the tested population, forward selection was generally superior to backward selection, indicating that phenotypic selection among the progenies is better than progeny-testing the previous generation.

More accurate breeding values can be estimated using information from relatives in procedures like combined index formation, e.g. combining family performance with individual performance or BLUP techniques. These techniques are able to maximize genetic gain when selecting a certain number of individuals, but at the cost of increased relatedness. This cost may be found acceptable in a breeding program of limited duration. Wei and Borralho (2000) found that group coancestry after unrestricted BLUP based selection in three trials with open pollinated progenies of Eucalyptus urophylla in southeastern China as less than 1 per cent, thus status number above 50, which is by no way problematic. However, the status number was reduced by a factor of more than 10 by the selection.

Gene Diversity in the Breeding Population Boosts Genetic Gain in the Production Population. Balanced selection may appear inefficient above. The aim of long term forest tree breeding is to supply forestry with best possible genetically improved regeneration material on a sustainable basis. High genetic gain and high gene diversity in the long-term breeding population are just different tools to achieve that goal. To get regeneration material, the breeding population is creamed off to achieve as high an immediate gain combined with an acceptable level of gene diversity. If the breeding population is more diverse, selection for the production population can be done more aggressively, sacrificing a higher share of the gene diversity in the breeding stock. Thus, a more diverse breeding population results in a higher gain in the step when the breeding population is creamed off, thus when selection for the seed orchard is done. This effect partly compensates for the faster advancement of gain in the long-term breeding population, which is possible by caring less about the accumulation of relatedness (Rosvall 1999). Thus it has been decided to use rather balanced designs in Swedish breeding. An implication for low-input breeding may be that a high number of parents is desirable for stands used for long-term population improvement than for commercial seed production. If open pollination families are used, that may not be expensive. To base breeding efforts on a wider genetic base than seed production means that a physical separation is needed between long-term improvement and seed production stands. If both are productive stands, such a separation may be easy and cheap. Long term improvement stands can be harvested for cones for two purposes:
Seed production areas (seedling seed orchards) where progeny (OP seeds) from say the 35 best trees are used to establish a stand;
Long term improvement stands where the selection intensity is lower and OP seeds from say 100 female parents are used.

Clone testing for sexual reproduction

As breeding strategies build on clonal testing are generally superior to unreplicated phenotypes, they should generally be preferred provided it can be done in fast, cheap and uncomplicated ways. It is evident and commonly accepted that clonal testing is important when the end use is clonal forestry, but it should be wider recognized that it often can be a superior strategy also when production forestry is based on seeds. Those seeds could be obtained from genotypes chosen by clonal testing rather than progeny-testing. Some species are as easy to propagate and handle as vegetative propagules as they are from seed, or vegetative propagation may actually be the only practical way to produce plants. In such clonal testing is very likely to have an important role. Clones are usually thought of as the ultimate tool for high gain, but clone testing may also be a key to low input breeding. If there are difficulties to multiply mature tested clones, that is a powerful argument against clonal forestry, but if sexual progeny can be obtained it is still worth to use clones for

To use clone plantations for seed collection has been suggested as a cheaper alternative to seed orchards of Norway spruce in Sweden (Lindgren and Karlsson 1993). Above it was argued that phenotypic selection often was a competitive alternative. Clonal testing instead of phenotypic testing can be seen as a way as increasing the heritability and thus the associated selection gain. This ought to be true even if when tested clones are unidentified, thus e.g. planting clonal rows and select the good rows for seed harvest ought to have potential for long term breeding or seed collection, even if the clones can not be identified or propagated vegetative.

For low input breeding, it is usually unrealistic to make large efforts to improve the prospects for vegetative propagation, but if the techniques are there it seems wise to use them instead of relying on phenotypic testing or progeny-testing.

Estimates of Accumulation of Relatedness Based on Fertility Variations

Low-input programs must be concerned with inbreeding, relatedness and diversity. These factors must be predicted to manage gene resource plantations and to plan seed collection. In high-input programs, known pedigrees, individual identification and selection algorithms that utilize this knowledge can keep control. In low-input programs, the actual operational control may be relaxed, and thus it becomes more important to forecast what will happen by appropriate use of theoretical predictions and by reasonable estimates of key factors. What happens depends mainly on the gene pool of the population and variations in the contributions of individuals to the next generation. To predict what happens is an advanced operation. The likely consequences may be forecasted by simple heuristic rules, tables and instructions for less-advanced users, but as future low-input breeders will very likely have access to competence and computers, the need of predictions will seldom be bottlenecks.

The gene pool of the offspring is the same as the gene pool of the successful gametes of the parents; this connection links generations. It seems natural to link fertility to successful gametes, but it is not known what gametes will be successful in advance, and there will be stochastic variation, which is high if low-input measures are used. It is thus more useful to define fertility as a characteristic of the parental genotype. Fertility is defined as “a parent's ability to produce successful gametes”. The true number of successful gametes per parent is both technically and principally difficult (or impossible) to estimate (e.g. there is no unequivocal definition of how long the zygote that results from a successful gamete must survive to be characterized as "successful"). Quantitative estimates of variation among trees in female or male reproductive structures can be made even for a low-input program, and it seems likely that such counts will be sufficiently accurate for most situations. Anyway, the differences in fertility among considered objects or years are likely to be more important than the inaccuracies of the estimation method.

A quantification of fertility differences among a group of parents in probabilistic terms can be made as a basis for predictions and theoretical development. The sibling coefficient (Kang 2001) refers to the probability that two gametes, chosen randomly from the gene pool of gametes, originate from the same parent, compared to that in the gene pool of the parents. It is associated to the probability that individuals share the same parent, and thus are sibs. Mathematically, sibling coefficient, Ψ, can be defined as $N\Sigma p_i^2$, in which N is the number of individuals and p_i is the relative fertility of individual i. Relevant theory is developed in the PhD theses by Bila (2000) and Kang (2001) and papers in them. "The effective number of parents" can be expressed as N/Ψ, which can be viewed as identical to the classical “variance effective number”. The sibling coefficient is a function of the coefficient of variation for fertility; Ψ=2 corresponds to a coefficient of variation of 100%. Sibling coefficient for a forest stand may typically be 2 (Bila 2000), but much higher values has been observed in individual cases. Ψ=2 means that there will be twice as many sibs among the seeds as expected if all mating were equally frequent. Ψ=2 means that relatedness and later inbreeding will build up over generations twice as fast as if there were equal mating. Reasons to suggest “sibling coefficient” to be useful in predictions of generation shifts are that it is independent of the number of members in the population, that it focuses on probabilities and that it has transparent interpretations as mentioned above. An example of this calculation technique is demonstrated in Bila (2000). The loss over generations is predicted when seeds are collected and a small sample of these seeds is used to replace the stand (a gene conservation stand). If a limited number of offspring is considered, the successful gametes can be seen as obtained by sampling from all gametes. An option to increase the effective number, thus to reduce group coancestry, loss of gene diversity and subsequent inbreeding, is to keep female fertility constant by mixing the same amount of seeds from all trees. This is a rather effective measure. The technique can also be used to trade off gain and gene diversity in the seed crop or gain and gene diversity in the stand itself (low-input breeding). The idea is that inbreeding and the associated phenomenon can be kept manageable and balanced against cost, gain and other desiderata by management technique and numbers, rather than by keeping exact pedigrees. Such calculations are needed for a low-input program, but can be generalized in tables. There can be a tremendous variation in the magnitude of fertility variation. For example, Varghese et al. (2003) reported a sibling coefficient, Ψ=17.4 in a first generation progeny trial of Eucalyptus tereticornis in south India studied at four years of age with the intention to convert it to a seedling seed orchard. Only 18% trees were fertile out of 200 trees selected for phenotypic superiority. Most cases with high sibling coefficient are objects which are young or flower poorly (Kang et al. 2003) or are not well adapted or it happens to be a special year, and probably not important overall, and even the seeds from the object just described would not have dangerously low gene diversity.

Use More Offspring from the Best Parents

Increase in relatedness and breeding value are the major outcomes of selection, and there are optimal combinations of them in the sense that under given constraints and gene diversity, there exists an optimal strategy that maximizes gain. An optimal strategy may be conservative or aggressive depending on the demand of gene diversity. It is a good breeding practice to allow the better trees to be over-represented in both breeding populations and production populations. A more gradual differentiation in treatment of materials relative to their goodness is more optimal than the either/or truncation selection usually practiced in breeding. Sophisticated algorithms for identifying such strategies have been developed for deployment. Different variants of linear deployment has been suggested for deployment to seed orchards, candidate populations and the breeding population (e.g. Lindgren and Matheson 1986, Bondesson and Lindgren 1993; Wei and Lindgren 1995, Lindgren and Mullin 1997; Andersson 1999 Lstiburek et al 2005). Different ways of introducing such unbalances were reviewed by Lindgren (2005). While paternal fertility may be unknown, it is still possible to trade off against female fertility, and thus pick more seeds and plant more plants from the best female parents. Fernandez and Toro (2001) applied integer mathematical programming in a selection scheme on an open-pollinated population in Eucalyptus globulus, resulting in a large reduction in loss of gene diversity at a small cost of genetic gain compared to truncation selection. There seems to be a need to develop techniques that are simple to handle, but still close to optimal for low-input breeding. Many optimization techniques require plants to be identified by family within gene resource plantations; this is an argument for family identification in the field. These techniques will contribute a better guarantee that diversity is preserved and make the balance between gain and diversity more of a deliberate choice. I doubt it is efficient keeping identifications in a low-input program for this reason only, but if this is done for other reasons, then techniques using this knowledge could be used. Another application could be to consider the family value for low heritability characters or characters such as survival, as a guide to how many to select from each family, but to select phenotypically within family (preferable for other characters). To harvest cones from fewer trees than are left as possible pollen parents can be seen as using more offspring from better trees, the best trees will be used both as seed parents and pollen parents, while more trees will be used at least as pollen parents.

Acknowledgement

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Seed set for Scots pine grafts is difficult to predict

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Extended abstract

Having predictions for breeding values of clones for both seed set and the genetic quality of the seeds, these two desirabilities may be balanced when choosing clones for a seed orchard (Lindgren et al 2004), but to do that we must know more about the genetics and predictability of seed set.

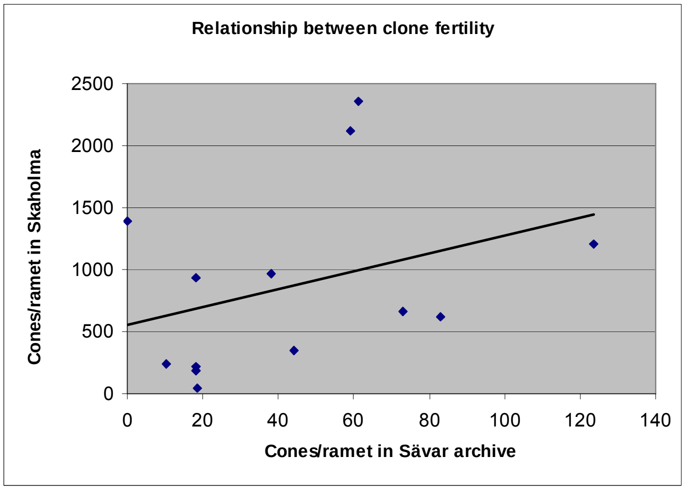
The study is based on predicted seed production for Scots pine grafted clones in Sweden with origin north of about latitude 62 (the four northernmost counties). Half a century ago plus trees were selected in mature autochthonous forests. At selection comments on the cone-set of the plus-trees were noted. The plus-trees were grafted. An archive with grafted clones of almost all selections (748) plus tree clones) was established at Sävar (present SkogForsk Umeå station close to the coast at latitude 63°54' and altitude 10m) in 1969-70. For each clone a row with four grafts was planted. Many of these clones have also been used in seed orchards and trials. The cone set of each ramet in the clone archive was screened 1984, the information collected was mainly intended to guide when selecting clones for seed orchards. Few clones had few cones, about 7% had less than 25 cones, just a few percent had more than 1000 cones, the average was 120 cones/ramet. The coefficient of variation among clones for cone-set was 94% and for ramets within clones 68%, thus cone-set was rather variable. The northern-most clones originating about 300 kms north of the archive had about half as many cones and they had also a lower diameter growth, but this is not sufficient explanation for the lower cone-production.

The female fertility for some of these clones has also been observed in four seed orchards and a clone trial. These observations of female fertility were compared with the earlier observations of cone set. The observations are compiled. The observations often do not include all clones in a seed orchard and comments were not made for all plus trees, thus it is only for some clones pair of observations exists.

Object	Lat	Alt	Age	Number of observation pairs (clones)		Correlation	Weight[B]
Seed orchard		M	Years	Archive	Plus trees	Archive - object	
Skaholma	63°50'	5m	43	13	11	0.35	2
Robertsfors	64°	40m	20	30	20	0.22	3
Klocke	62°54'	75m	21	12	12	0.06	2
Sävar SO 99	63°54'	10	30	20	18	-0.21	3
Clone trial [A]				10	8		
Sävar 82	63°54'	10	12			-0.44	0.5
Sävar 93			23			0.10	1
Sävar 04			34			-0.05	1
Röskär	59°25'	25m	21			0.00	1
Degeberga	55°47'	120m	22			0.29	1

[A] The clone trial was planted at three localities in northern, middle and south Sweden. The northern site was measured at three occasions

[B] Subjective weight for weighted average



The relation between observations prior to selection and in a mature seed orchard is illustrated by the figure (Skaholma was the object with the highest correlation).

For each object a correlation was calculated. To visualize what potential gains in female fertility which may be possible also a predicted percentage potential gain was estimated. It was assumed that the selections raised the cone set in the archive or plus-trees by one standard deviation (selection intensity = 1, which is an unrealistic high weight given to cone-set for seed orchard applications). Averages are compiled in the table below. The female fertility observations in the objects differ, it may be cones, seeds or female strobili, and the quality of the information differs (number of ramets observed, accuracy of method, age of grafts at observation). Therefore for gain predictions also a weighted average where the objects are given subjective weights (see table above) was calculated.

	Archive	Plus trees
Correlation – mean	0.081	0.055
Gain potential – mean	-2.6 %	-4.2 %
Gain potential –weighted mean	8.9 %	-5.1 %

The comparisons suggested correlation close to zero between evaluations of cone set in plus-trees or clone archive versus cone set or seed set of clones in seed orchards. In no single case out of 18 comparisons there was a significant relationship between cone-set predictions based on observations in the archive or plus trees and female fertility observed in seed orchards or trials for the same clones. The predicted gain in seed production by choosing clones with a good cone set as plus-trees or a good cone set in the graft archive was low and uncertain. It is noted that the two lowest correlations are between objects at Sävar, which grow only a few hundred meters from each other. In the clonal trial the same clones have been observed at different locations and different years, but the observations did not seem to correlate well.

This study may be an argument against locating seed orchards many latitudes south of origin of the clones. Skaholma is such a seed orchard (it is around 20 kms from Sävar but the clones origin from around 300 kms from the north), and the figure shows that only one of the clones is above average in the clone archive and half of them are among the poorest at the clone archive. But still a very high seed production was registered in this seed orchard 2004 (based in the same object data as this study, Prescher et al 2005)!

This is a limited study. It is for Scots pine grafts in northern Sweden. A single archive which was not meant to function as an experiment was inventoried a single year when the grafts had not got old enough for full cone production. Thus generalizations of this study would require observations under more circumstances.

This study suggests that no efforts should be done to predict female fertility for orchard candidates. This simplifies selecting efforts and low-input breeding.

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Keywords: Cone production, female fertility, seed orchard, *Pinus sylvestris*

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Lessons from 50 Years of Tree Improvement with Loblolly Pine in the NC State University Tree Improvement Program

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Abstract

As "experts" in tree improvement with loblolly pine (*Pinus taeda* L.) in the southeastern United States, we know how to make mistakes! Fortunately, during the 50 years of tree improvement with the Cooperative Tree Improvement Program at NC State University, we have also had some great successes. Although ours is a very large and intensively managed breeding program, the members of the Cooperative insist that we are as efficient as possible in our breeding, testing, and selection efforts. The 3rd-cycle breeding strategy that was adopted almost 15 years ago emphasized the need to devote resources to efforts to maximize short-term economic returns but also maintain genetic diversity in the large breeding populations.

Fortunately the deployment of genetically improved propagules is rather straightforward with loblolly pine. Genotype by environment interaction (GxE) is generally not a significant concern with OP, full-sib or clonal material, so numerous breeding regions and deployment regions are not needed. This simplifies many breeding decisions and keeps costs reasonable.

Production of propagules of loblolly pine for regeneration is both extremely easy and cheap as well as complex and expensive. Nowhere in the world are bareroot seedlings produced as efficiently as in the US South. Seedlings derived from advanced-cycle loblolly pine seed orchards typically sell for \$0.045 to \$0.055 USD each. Regeneration success with these inexpensive seedlings is typically very high. Clonal propagules that are the highest genetic quality seedlings are about 7x to 9x more expensive than bareroot seedlings. A challenge to tree breeders is to produce propagules with sufficient genetic quality to warrant the extra cost of producing these clonal propagules. We will emphasize these and other experiences from our breeding program to illustrate how costs can be kept manageable for programs of any magnitude.

Keywords: Breeding, *Pinus taeda* L., selection, tree improvement, silviculture

Improving Wild Cherry (*Prunus avium*), Wild Pear Tree (*Pyrus pyraster*) and Wild Service Tree (*Sorbus torminalis*) by combining progeny testing and micropropagation

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Abstract

The demand for high value timber of wild cherry (*Prunus avium* (L.) L.), wild pear (*Pyrus pyraster* (L.) BURGSD.) and wild service tree (*Sorbus torminalis* (L.) CRANTZ) increased during the last decades in Germany and other European countries. Wild cherry is a rare species (below 0.5 % of the forest area), while wild pear and wild service tree belong to the rarest forest trees in Central Europe which occur scattered as single trees or small groups. Their survival can be forced by conservation and utilization. Forest owners are much more interested in planting species which promise a high return of their investment than in planting trees just for conservation purposes. Since seed of the three species is scarce and often of low quality due to lack of pollination partners or contamination with pollen of fruit varieties the Northwest German Forest Research Institute started an improvement program by means of vegetative propagation. In order to achieve a maximum genetic gain in short time, the ortets were not selected in natural stands but in existing progeny trials. For establishing single-tree progeny trials seed was collected from selected plus trees and seedlings were grown separately in the nursery. Field trials were established with those half-sib families which gave rise to vigorous plants in sufficient numbers. After evaluation of quality and growth parameters of the tested progenies so-called elite trees were selected in outstanding families as ortets for micropropagation. Micropropagated clones were planted in clonal field trials in comparison to seedlings. Cryopreservation protocols are being developed in order to store in vitro material for the duration of the clonal field trials. In wild cherry, clones which are significantly superior in at least one important character to seedlings without being significantly inferior in other important characters are certified and registered as tested clones according to the German law of Forest Reproductive Material. Only certified clones are permitted to be commercialized. *Pyrus* and *Sorbus* are treated in the same way although they do not come under the mentioned law.

Keywords: high value timber, rare species, conservation, tissue culture, selection, clonal trial, cryopreservation

Low – Cost Breeding and Gene Conservation of Norway Spruce in Romania

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Abstract

Norway spruce is the most important coniferous species in Romania, covering 23 % of national forest area and 78 % of the coniferous forests. That is why Norway spruce breeding has started approximately five decades ago by study of the genetic variability at the population level and selection of the most valuable natural populations and designed them as seed stands. There were installed 3 experimental series in 1968, 1972 and 1984 totalising 17 long – term comparative trials. In these trials there are tested 128 geographical provenances of which 50 from Romania and 78 from 13 European countries: Norway (5), Sweden (6), Finland (13), France (10), Switzerland (8), Germany (6), Austria (14), Italy (3), Poland (3), Czech Republic (2), Slovakia (1), Hungary (4), Bulgaria (3). The research results proved that at 30 years old, there is a great interpopulational genetic variability for the most studied traits. The wood output that can be obtained by selection of the most valuable local populations is between: 8 – 47 %. Regarding the growth and adaptive performances, the most valuable provenances are from Herciniano – Carpathian and South – Alps areas (Romania, Czech Republic, Slovakia and Austria). Provenances from Scandinavia, Bulgaria and high-elevated populations from the Alps differentiate clearly from the rest of provenances, obtaining the least results. No significant provenance x environment interaction has been obtained for growth traits, thickness of branches and stem straightness; but it is important for survival and resistance of biotic and abiotic factors. The growth traits have also a higher stability in space and time then the other studied traits. As the genetic value of the provenances has been tested by means of researches, the best Romanian provenances will be designated as "tested seeds sources" for the provenance regions where there are established the trials, in conformity to OCDE Scheme and national legislation in this field. Therefore the provenances trials are not only a low – cost breeding method but also they are *ex situ* forest genetic resources, that provide numerous and important information for scientific point of view and for conservation of forest genetic resources.

Keywords: *Picea abies*, genetic variability, provenances, forest genetic resources

Breeding Strategy for Douglas-fir in Spain

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Abstract

In 1978-81 a series of Douglas-fir provenances trials were planted at 16 locations in northern Spain. Some results obtained in those trials, including the relative importance of variance components (especially the provenance by site interaction), the low provenance mean heritability for growth rate in many sites, the lack of high quality sites in our trial, and the fact that provenance performance tended to become more stable with time, hindered the definition of a long-term breeding strategy. We discuss the reliability of the provenance selection results from the unbalanced, incomplete allocation of provenances to sites, and show the consequences of this in continuing the second phase of the breeding program. An effective and economical program based on individual tree selection across the provenance trials from a full range of provenances is proposed. For the production population, individual selection across the provenance trials is an acceptable strategy We propose to include timber stiffness in the breeding objective and utilise density and sound velocity as selection criteria to make new individual tree selections. For the breeding population we propose the creation of inter-provenance hybrids by poly-crossing. The idea is to utilise as female parents 55 genotypes from the clonal archive crossing with two different pollen mixes with 30 male parents per mix, selected by timber stiffness at the trials. A total of 110 polycross families will be our breeding population for forwards selection to create a new future breeding population, and will enable estimates of breeding values of female parents to be used for backwards-selecting clones for new orchards. Further generations will be enhanced by selections from other breeding programmes (e.g. New Zealand, France) in the form of pollen in the poly mixes to increase status number.

Keywords: inter-provenance hybrids, wood quality selection, *Pseudotsuga menziessiii*, breeding population

Populations Strategies with Low Input Breeding

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Abstract

Formally, Forest Tree Breeding has been focused on large programmes with few major economic species. Now, with increasing interest in biodiversity and in genetic conservation, many minor temperate or tropical species are coming into consideration. How to balance costly advanced breeding programmes with few species versus cheap programmes with many species? To meet this new challenge, this paper does proposals for cheap compact though rather still efficient programmes. Forest Tree Breeding includes successively: Provenance testing, Seed Stands selection and Individual selection. Here are following two main cheap strategies based on populations ensuring genetic conservation as well (NANSON, 2004). A) At P**rovenance testing**: 1) determine the best probable ones for the considered breeding region, (2) plant them separately, better with archiving, 3) select among them the best Seed Stands, 4) collect seed for plantations on numerous best trees (B3). For further generations, skip to B2). Option: 1) collect many supposed good provenances or varieties (e.g.: 20-40), 2), mix the seed, 3) plant, thin selectively into a Seed Stand. Another option: 1) sow the provenances separately, 2) label all plants and plant them (better in one-tree plots), thin them selectively into a "Provenance Seedling Seed Orchard" with a very large gene base. B) **Seed Stands**: (1) subdivide the country into Regions of Provenance, (2) select Seed Stands (OECD or European Union Scheme), (3) collect seed on at least 30-50 (but better on 200-300) well spaced superior trees, (4) plant and when possible archive, (5) resume with the best new Seed Stands. Option: 1) mix seed from many good Seed Stands of the same Region of Provenance, 2) plant them, 3) thin selectively into new Seed Stands for next generation, etc. When properly done with an adequate number of good seed trees, this population strategy ensures gene conservation in the long run of best provenances or stands. It has been recently proposed by the author the conserve the magnificent Seed Stand of beech of "Forêt de Soignes" (Brussels) from 200 to 300 selected sub-plus seed trees.

Keywords: populations, strategies, breeding, conservation.

Introduction

In the last decades, Forest Tree Breeding has been focused on some major economic species for which large and costly programmes have been developed. This is the case for example with loblolly pine (*Pinus taeda*), slash pine (*Pinus elliottii*), Douglas fir (*Pseudotsuga menziesii*) in the United States of America, *Pinus radiata* in New Zealand, Scots pine (*Pinus sylvestris*) in

Scandinavia, Norway Spruce (*Picea abies*) and Douglas fir (*Pseudotsuga menziesii*) in Western Europe, Maritime pine (*Pinus pinaster*) in France.

On the contrary, the other species even with large economic interest as many hardwoods were neglected, as for example oaks, beech, maple, wild cherry in the United States or in Europe. Furthermore species with little or no economic value were in general quite forgotten, as birch, alder, *Sorbus*, wild apple or pear tree, etc. In these last species some problems of genetic conservation could sometimes arise too.

In the tropics where the number of forest species is much higher, the situation is still worse. Forest Tree Breeding is restricted mainly to some major species as tropical pines, eucalypts and some acacias, the numerous other species being ignored, even when reaching a certain economic interest.

With the rise of the ecologic movement, the focus turned toward biodiversity (though often in an excessive way) and to genetic conservation. This last item became even more important to deciders and funds providers than Forest Tree Breeding itself.

How to meet this challenge in a reasonable way?

Must we continue with costly advanced breeding programmes with a few species or must we start with many small and cheap programmes including many species and taking genetic conservation into account? If so, what can be these cheap programmes?

To debate this issue is the purpose of this paper.

Methods

For the sake of clarity, let us remind that Forest Tree Breeding includes in principle three successive phases: Provenance testing, Seed Stands selection and afterwards Individual selection.

1) Provenances testing

In our mind, this is the first step for improving a given species. As a matter of fact, provenances testing which was mainly developed in Europe on many species, showed large variation among provenances. Surprisingly, it showed that the local material was not always the best, neither in production nor even in adaptability. As a consequence, to start individual selection with the local material, even autochthonous, can be misleading because some other provenances can be superior to the local breeds obtained often after a long and costly individual breeding programme. Such situations occurred in South Sweden and in France with Norway Spruce.

The genetic gain in total production due to provenance testing and to the selection of the best ones for afforestation rather than using averages provenances (or random ones), varies according to species and situations but can be evaluated in average roughly to 20 % (NANSON, 2004). Other gains on quality (form, etc.) and resistance to pests and adaptability are usually obtained as well.

However, this stage of provenances testing takes usually some tenths of years (at least 15-20 years) and some breeding programmes, consciously or not, have started directly with the second phase (Seed Stands) or even with the third one (Individual selection).

Finally, let us note that gains from provenances testing are not necessarily a "one shot" gain because provenances testing is often made through different stages approaching successively to the out-best provenances as showed with Douglas fir provenances testing in Europe (now located in the J-shaped area in the Washington State).

Provenance testing does not require a staff of advanced geneticists to be performed once good long-term experimental designs are properly used.

2) Seed Stands selection

Once the best provenances and Regions of Provenance for a given "Breeding region"[1] are found, selection of "Seed Stands" (superior stands) may begin inside the best Regions of Provenance.

This selection is made on the basis of the productivity of the stands, their quality (form, branching, etc.), their the resistance to pests and their adaptability. Other criterions are also taken into consideration as the effective number of flowering trees (as possible some hundreds), the isolation from inferior sources of pollen, etc.

Genetic gains in total production due to Seed Stands selection and their use for afforestation though variable as well, can be evaluated in average roughly to 5-10 %. Other gains on quality, resistance and adaptability are usually obtained too. For example in hardwoods, the gains are more directed toward quality improvement than toward productivity. These gains are adding to that of provenances selection when Seed Stands are selected among the best regions of provenance.

This second phase can be carried out within some years and at low costs by a small team of foresters supervised by a forest geneticist able to integrate all knowledge on heritability of different characters. The author is among other things a specialist of this Seed Stands selection.

3) Individual selection

In principle, for a given breeding region, this phase of individual selection may only begin after the two preceding ones, i.e., within the best Seed Stands of the best provenances for this breeding region. For recall, Individual selection includes "Plus trees" selection, setting up of "Plus trees Seed Orchards", progeny testing (very long and costly) and setting up of "Elite trees Seed Orchards" (Figure 1, NANSON, 2004). Finally, Seedlings Seed Orchards and clonal varieties, as "multiclonal varieties" can be produced from the best progenies of the Seed Orchards or from the best crossings (Figure 2).

This phase is considered as the most accomplished phase of tree breeding. Genetic gains on productivity are also variable but can amount to 5 to 20 % according to the type of Seed Orchard (first generation) and possibly to an additional gain of 10-20 % for (multi)clonal varieties. Gains on quality are probably higher. When made from the best Seed Stands of the best provenances, gains from individual selection are adding to the preceding ones.

However, this phase is very long, costly and requires staffs of high-level geneticists. It can be continued over many generations but with the growing challenge of diminishing gains and increasing inbreeding with therefore increasing costs par unit of gain.

Costs of genetically improved plants produced by vegetative propagation (multi)clonal varieties) are particularly high: usually at least more than twice the cost of seedling plants what could discourage foresters.

4) Combining Strategies

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[1] **Breeding region**: a sufficiently homogeneous region where given provenances or varieties can be grown with the same success.

The present possible general Breeding Strategies of recurrent selection over many generations combining the former 3 main phases are summarised by figures 1 and 2, extracted from NANSON (2004). They will help to clarify options taken in the next chapter (proposed strategies).

A) Before 1960 (Figure 1)

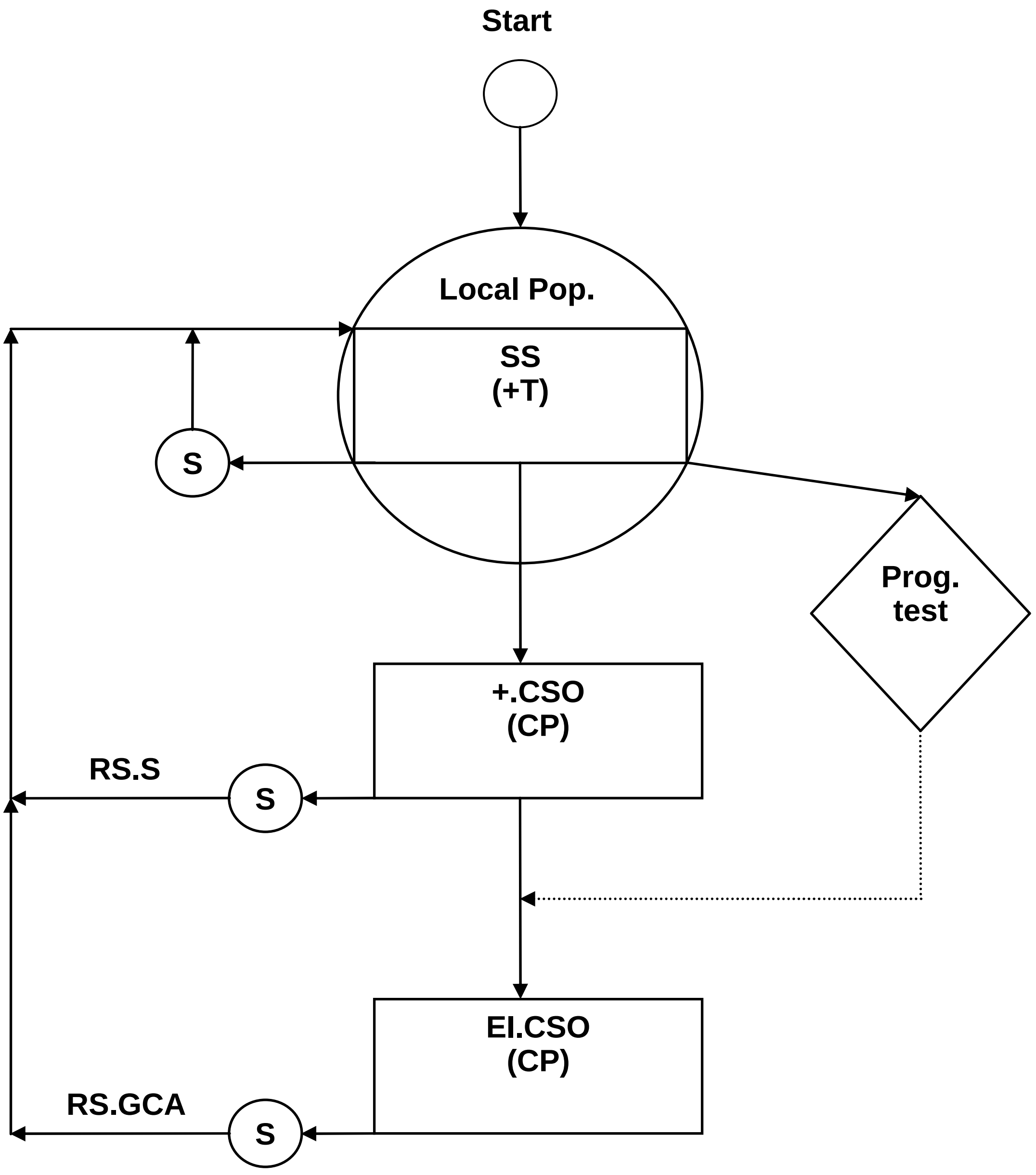
Figure 1 displays the diagram of a complete breeding strategy before around 1960 when the local material was considered as the best possible one. It shows two main possible strategies.

a) The first one is to select "Seed Stands" (SS) within local populations (considered as the best), collect seed (S) on them for afforestation. At least a part of these plantations is being archived for the selection of Seed Stands at the next generation. And over again, on an indefinite number of generations... This could be called a "populations simple recurrent selection" based on the aggregate phenotype of populations.

b) The second one is the classical "Individual selection" with selection of "Plus trees" mainly in the Seed Stands, setting up of "Plus trees clonal seed orchards" (+.CSO) and after "Progeny testing" setting up of "Elite tree Seed Orchards (El.CSO). These two types of Seed Orchards are producing seed for afforestation. These new stands, as far as possible archived, are providing new "Plus trees" for repeating the cycle.

This can be repeated regularly over generations as respectively "Simple recurrent selection" (RS.S) based on individual phenotype selection or "Recurrent selection for general combining ability" (RS.GCA) based on individual genetic selection.

Figure 1.- Diagramme of a complete breeding strategy before 1960



Legend

SS : Seed stand
+T : Plus tree
+.CSO : Plus tree clonal seed orchard
El.CSO : Elite tree clonal seed orchard
(CP) : Corresponding Clonal Park

← Flow of
←┈┈ Flow of

B)

After 1960 (Figure 2)

At that time, Provenance experiments showed that the local material was not necessarily the best for afforestation.

a) Detection of the best provenances had to be done through Provenance experiments (Prov or Var Test) prior to Seed Stands selection and Individual selection. Once detected, seed (S) of these best provenances can be bought for afforestation in the considered Breeding region.

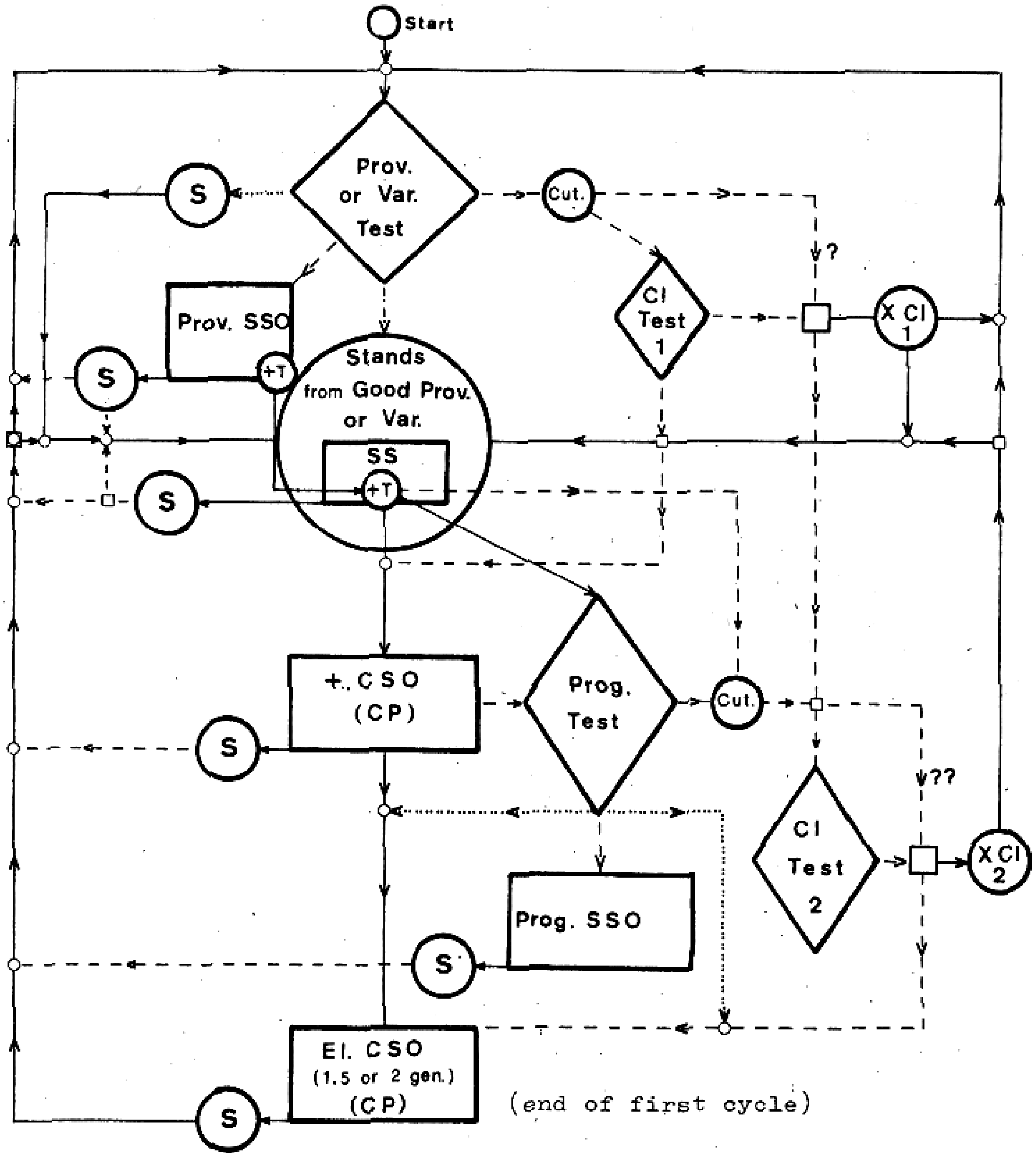
b) At next generation, the best stands originating from these provenances, as far as possible archived, can be selected as Seed Stands (SS) providing seed (S) for afforestation, as seen earlier.

c) An intermediary phase can be developed through "Provenance Seedling Seed Orchards" (Prov. SSO) (NANSON, 1972). This last is similar to a "Progeny Seedling Seed Orchard" where progenies are replaced by the best provenances (or varieties) known to date. It is a provenance test with numerous small plots (ideally one-tree plots) submitted to strong selective thinnings leaving only the best-adapted trees to the considered Breeding Region.

d) The classical Individual Selection can then take place with "Plus trees" from "Provenance Seedling Seed Orchards" and from "Seed Stands" similarly to the scheme of Figure 1. However here, "Progeny Seedling Seed Orchards" (Prog. SSO) can be this time developed from progeny tests (Prog. test) through adequate selective thinnings.

e) Selection of clones for vegetative propagation can be developed from provenances or variety tests or better from progeny tests. It gives rise, through Clonal Tests (Cl Test 1 or 2) or not, to (multi)clonal varieties (XCL).

Figure 2.- Diagramme of a regular general breeding strategy for a single species.



Legend.

SS: Seed stand
+T: Plus tree
+.CSO: Plus tree clonal seed orchard
El.CSO: Elite " " " "
(CP): Corresponding clonal park
Prov.SSO: Provenance seedling seed orchard
Prog.SSO: Progeny " " "
(S) Seed for afforestation
(XC1) Multiclonal variety

(Cut) Cutting
——→ flow of material
– – → optional flow of material
········→ flow of information
–·–·→ mixed flow
○ one way branching
□ two way branching
Square or rectangle: production units
Rhomb: sequential test

proposed strategies

With necessarily limited resources, how to balance costly advanced breeding programmes with a few species versus numerous cheap programmes with an increasing number of species that needs more and more consideration?
The answers to this challenge and their justifications have been described with details in the chapter 5 (Strategies and breeding programmes) of my book (NANSON, 2004).
First, if advanced breeding programmes on major species are under way, it seems wise to continue them at least for the current generation (cycle).
Second, for the reasons cited in the introduction, a growing amount of resources should be attributed to other secondary or minor species for breeding and possibly genetic conservation.
Because of the large number of these species, cheap strategies are necessarily requested.
Here are following two main cheap breeding strategies centred on populations with large genetic biodiversity, based on a combination of Provenances testing and of Seed Stands selection. These strategies are ensuring genetic conservation as well.
These strategies are given mainly under the format of brief receipts, their justifications being given before, or in NANSON (2004).

A) Strategies mainly based on Provenances testing

Main procedure
Let us consider a Breeding Region (circle in Figure 2) where provenances testing has been performed on one or two phases. Certain provenances are found as the best in main respects.
Seed is imported from these provenances and commercial stands are planted separately by provenance. At least a part of them is archived.
Among these last stands, the best are selected, thinned selectively and admitted as Seed Stands in the National or regional Catalogue of Basic Materials (OECD, 1997, European Union, 2000).
Seed is then collected on these Seed Stands and used for afforestation. On each Seed Stand, seed collection takes place at least on 20 to 50 (better on some hundreds: 200-300) of well spaced and marked good trees, for example on sub-plus trees (= almost Plus trees). This, in order to preserve genetic diversity and prevent inbreeding in the long run within every population.
For further generations, the same scenario can be resumed at the level of archived plantations giving rise to new Seed Stands.
In principle, this option is rather cheap and can be performed by foresters helped by consultants in Forest Genetics.

Option 1.
1) Collect many supposed good provenances or varieties (e.g.: 20-40).
2) Mix their seed thoroughly.
3) Plant the derived plants on rather homogeneous soil plantations and archive them.
4) Thin them very selectively and after some such thinnings and finally a good-looking overall phenotype, transform this plantation into a Seed Stand.
5) As said earlier, collect commercial seed on at least on 20 to 50 (better on some hundreds: 200-300) of well-spaced and marked good trees, for example on sub-plus trees.
6) Use this seed for commercial plantations and archive some of them.
7) Etc., at he level of this new synthetic population.

This option can be performed by foresters helped by consultants in Forest Genetics. It is very cheap and performing providing a population with a very high genetic diversity able to adapt itself easily to various environments. A drawback is the lost of the provenance identity of the future selected plus trees for further possible Individual selection.

Option 2.

1) Sow the provenances separately.
2) Label all plants.
3) Plant them (in a statistical design, better one-tree plots), on rather homogeneous soil plantations and map them plant per plant.
4) Measure them individually and perform analysis of variance/covariance of individual traits (see NANSON, 2004, chap. 5 & 2.6.4) with possible index for selection.
4) Thin them selectively into a "Provenance Seedling Seed Orchard" with a very large gene base (NANSON, 1972).
5) Collect seed as said earlier for commercial plantations.

For further generations, the scenario can be resumed at the level of the recurrent provenance/variety tests.
This option needs a staff of high-level geneticists mainly to do the adequate selective thinnings based on analysis of variance/covariance leading possibly to Index and to maintain the Provenance Seedling Seed Orchard. It is therefore more expensive. But it conserves the identity of future selected trees for further possible Individual breeding.

B) Strategies mainly based on Seed Stands

Main procedure
Many aspects of this strategy have been already described above (re-see also Figure 1). Once the local populations are proved or considered as the best, or in the extreme if no provenances testing is existing but that time is lacking and that a programme has to start rapidly (not recommended), the following procedure can take place for a given country or region.

1) Subdivide the country into "Regions of Provenance" (see OECD, 1997 or UE, 1999 schemes that are almost equivalent).
2) Select Seed Stands (idem).
3) Thin them selectively to the best trees.
3) Within Seed Stands, collect commercial seed as said above, on at least on 20 to 50 (better on some hundreds: 200-300) of well spaced and marked good trees, for example on sub-plus trees.
4) From the seed of every Seed Stand, make plantations in the adequate breeding region, and archive a part of them.
5) Resume with the best Seed Stands of this new generation.

For further generations, the same scenario can be resumed at the level of these archived Seed Stands. So, a "population simple recurrent selection" can take place. When properly done with an adequate number of good seed trees at every generation, this strategy ensures gene conservation in the long run of the best initial local stands.

Option

1) Mix seed from many good Seed Stands of the same Region of Provenance.
2) Plant them.
3) In adequate plantations, after selective heavy thinnings, make Seed Stands (in principle genetically all alike when on the same type of site) for the next generation, etc.
4) For further generations, collect commercial seed as said above, on at least on 20 to 50 (better on some hundreds: 200-300) of well spaced and marked good trees, for example on sub-plus trees.
5) Etc.

When properly done with an adequate number of good seed trees, this strategy ensures gene conservation in the long run of the mixture of the best local initial Seed Stands.

3) An example of application for a stand

The magnificent Seed Stand of beech (*Fagus sylvatica)* of "Forêt de Soignes" (nr 5BB0155) in the Brussels Region (Belgium), called sometimes the "cathedral forest", is getting old (more than 200 years) and approaching the dead line of 300 years considered as the extreme survival age of the beech. The global warning is exerting an additional stress on the older trees resulting sometimes in their progressive dye back.

This stand reaches a dominant high of 40-45 m, with trees up to 50 m and its productivity lies between 7 and 11 m^3 ha^{-1} ye^{-1}in total production what is, to the author knowledge, the highest in Europe. In Belgium, its progeny is the best in growth and in late flushing ensuring little late frosts even when planted in the open.

To save this remarkable Basic Material, 200-300 Sub Plus Trees are now being selected and marked all over this stand of 380 ha (NANSON, 2006).

In the future, seed will be collected under all these flowering trees, thoroughly mixed into one homogeneous seed lot to be used for afforestation in the Brussels region, in order to restore this magnificent forest and Seed Stand.

And this procedure can be resumed over the next generations, according to strategy B for a single stand.

Discussion

Many particular points have been already discussed.

More generally, at the extreme, it is necessary to compare the opportunity of a multi-species strategy possibly little advanced, against an advanced generation breeding strategy for one major species. Presented in other terms, when a breeding programme of a given major species is already advanced, is it not better at a certain time to commence the soft breeding of new species? This, instead of continuing the breeding programme of the main species whose advanced last cycles are more and more expensive, with decreasing gains and increasing inbreeding?

In fact, most breeding experiment stations have to deal with many species at the same time, as in Belgium.

Furthermore, the present ecological movement urges to consider much more species than before (even minor hardwoods). In this case, programmes have to be more limited, for example restricted to the selection of populations, and even to the conservation of the best ones.

In this multi-species strategy, we believe that a few priority species have to be bred rather far during the first cycle and even further on. At the same time for the other species, simple breeding programmes for example limited to the population selection and to the conservation of the best ones have to be developed. In this manner, the breeding optimum for the whole set of species within a Breeding region must not be far.

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Selection of *Pinus Pinea* L. Provenances Using Growth Models

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Abstract

The natural range of the European nut pine involves the occidental Mediterranean basin and the South European Atlantic coastal zones. In Portugal, European nut pine is one of the most important forest tree species, covering a non contiguous area of about 78 000 ha. In order to provide a breeding program for this species a provenance selection was undertaken and a field trial established. The objective of this study was to analyse the possibility of using growth models to support the selection of the best provenances using growth models. The advantage of using growth modelling to study the evolution of provenances is the possibility to compare growth rates. In fact growth rate is a way of measuring the species adaptability.

Keywords: *Pinus pinea* L., Provenances, Growth Models, Total Height, Height Growth Increment

Materials and Methods

The provenance trial was established in 1993 and is located in the "Perímetro Florestal de Sines" with a latitude of 38º01'N, longitude of 8º42'W and altitude of 228m. Twenty five provenances from the Mediterranean Basin were used: Portugal (P9, P22, P11, P10, P5), Spain (E23, E25, E24), Italy (IT4, IT18, IT6), Greece (G2, G3), Morocco (M14, M20, M1, M7), Turkey (T16, T21, T19, T17, T12, T15), and Israel (Is13, Is8). Each provenance is composed by twenty five different trees per plot, repeated for six randomized complete blocks planted with a 3 x 4 m spacing, resulting in a total of 3750 trees, occupying an area of about 4.5 ha.

The total height was measured at six different ages (2, 6, 7, 10, 11 and 13). Only the central trees of each plot were measured [1]. Height growth was modelled with growth functions formulated as difference equations [9]. This method allows the prediction of total height for each age, from the values observed at any other given age:

$$f(y_2) = f(y_1, t_1, t_2)$$

where y_2 is the value of the dependent variable (height) at age t_2 and y_1 is the corresponding height at age t_1. .Difference forms of Richards, Lundqvist-Korf and McDill-Amateis growth functions, widely used in forest research, were tested and fitted using the PROC NLIN procedure of SAS software [8]. As the data available refer to very young ages, the asymptote was not estimated with regression but else given the value of 25 m that was estimated as a reasonable value for the species, according to empirical knowledge [5]. Data used was structured to include all possible growth intervals. A total of 1350 pairs of age-dependent variables (t_i,y_i) were considered. According to Goelz and Huang [4, 6] this data structure provides the most stable and consistent results.

The functions will be mentioned as: Richards-A, Richards function where *A* is the free parameter (*k* or *n*) of the difference form, Lundqvist-A, Lundqvist-Korf function where *A* is the free parameter (*k* or *n*) of the difference form and McDill is the McDill-Amateis equation.

Candidate growth equations and afterwards selected growth equations for all provenances were chosen according to several statistics that allow the assessment of model fitting and predictive ability. The quality of the fitting was analysed through the R^2_{Adj}. The predictive ability and bias were evaluated using statistics computed with the Press residuals: mean of Press residuals (*MPress*) for bias evaluation and mean of the absolute value of the Press residuals (*MAPress*) to evaluate model precision [7].A summary statistic *TV* [3] that sums up, for each model, the normalized values of R^2_{Adj}, *MPress* and *MAPress* obtained for each provenance was used to help in the selection of the final model. Once the best growth equation was selected, the study of the influence of each provenance on the height growth model was done through dummy variables defined for each provenance.

Results and Discussion

Convergence was easily obtained for all candidate functions - except for the Richards-A equation. The Richards-m and McDill-Amanteis equation were discarded because, respectively of the low fitting ability that was achieved and the low asymptote value (*A* parameter). The Lundqvist-A, Lundqvist-k (with *A* as fixed parameter) and Richards-k (with *A* as fixed parameter) equations were selected because they presented the better fitting and prediction ability when fit to the whole data set.

The results obtained by fitting the three growth equations, for each provenance, were evaluated applying the *TV* statistic [3]. According to this statistic Richards-k model (equation 1) was the one with less *TV* value for each provenance, thus being chosen.

$$h_2 = 25\left(1-\left(1-\left(\frac{h_1}{25}\right)^{1-n}\right)^{\frac{t_2}{t_1}}\right)^{\frac{1}{1-n}} \quad \text{(Equation 1)}$$

where h_i is the total height (m), t_i is the age (years) and *n* the parameter. The results obtained by fitting the Richards-k equation with the parameters expressed as a linear function of the dummy variables for provenance, led to the classification of the provenances in three groups according to the similarities of growth curves. Table 1 shows the three provenance groups obtained, and their corresponding *n* value, provenances list and model statistics.

Table 1. Provenance groups obtained with Richards-k (with *A* as fixed parameter) equation and their corresponding *n* value, provenances list and model statistics.

Group	***n***	***Provenances***	***Mpress***	***MApress***	R^2_{Adj}
1	0.2270	E26, E27, G2, IS8, IS13, IT6, IT18, M7, P9, P10, P11, T11	0.072193	0.23131	0.89620
2	0.1821	E25, IT4, M1, M20, T12, T17, T19, T21	0.072891	0.22742	0.89428
3	0.1458	G3, M14, P5, P22, T16	0.069188	0.21416	0.88336

Group 1 joined provenances that are expected to achieve a higher growth rate as it is indicated by the highest *n* parameter value (*n*=0.2270). This group includes 12 out of the 25 provenances. On the other hand, provenances G3, M14, P5, P22 and T16 belong to the group which presented the lower *n* (*n*=0.1458) which represents the provenances with lower adaptation in this field site. Finally, group 2 joined eight provenances, such as E25, IT4, M1, M20, T12, T17, T19, T21, it is considered an intermediate group for growth rate. The use of the selected growth model contributed to increase the knowledge of the 25 provenances height growth development as it enables to point out the group with higher growth rate that indicates higher adaptability. However, trees used in this study are still young to allow a definitive conclusion about the best provenances involved.

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Development of a commercial breeding program for *Pinus tecunumanii* in South Africa

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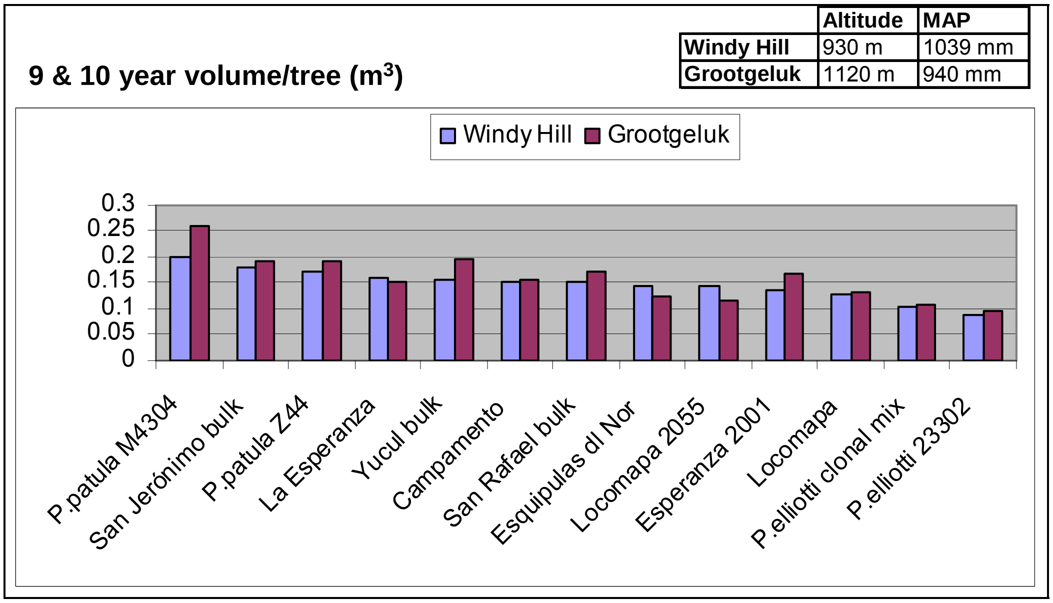
Abstract

Forestry in South Africa consists of an area of around 1.4 million hectares and these plantations cover around 1.2% of the total area of the country. During 2003, the forestry and forest products industry contributed around 1.2% to the GDP of the country. The species split is around 50% softwood (*Pinus*) species and the other 50% is made up of various *Eucalyptus* and *Acacia* species. The most widely planted softwood species is *Pinus patula*, followed by *P. elliottii* and then *P. taeda*. A large number of alternative pine species are being evaluated in the country through collaboration with Camcore.

Pinus tecunumanii was first identified in Guatemala in the 1950's as a species distinct from *P. oocarpa*. There are two distinct sub-groups in the species; populations that occur at low elevation below 1500 m (LE) and populations occurring at high elevation between 1500 and 2900 m above sea level (a.s.l.) (HE). It was introduced to Southern Africa by the Oxford Forestry Institute (OFI) in the 1970's and its genetic base expanded further through introductions by Camcore (North Carolina State University) in the 1980's and 1990's. Sappi started testing the species extensively in 1988 with separate trial series' of OFI and Camcore material, planted on sites covering a wide range of altitudes and climatic conditions. Some of the species' advantages in relation to *P. patula* are rapid site capture, improved drought tolerance, improved tolerance to the pitch canker fungus and better adaptability to warm sites. Disadvantages are sensitivity to frost and wet sites, upper-stem breakages, shallow root systems with resultant wind-throw problems, and shy seed production. The number of *P. tecunumanii* tests, provenances and families tested in Southern Africa are given in Table 1.

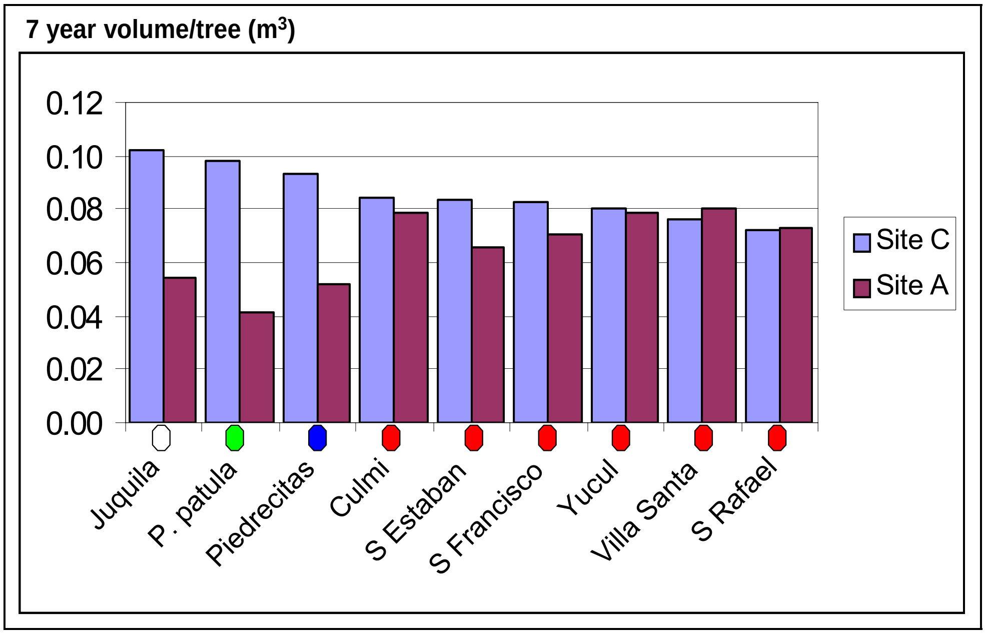
	No of tests			No of provenances tested			No of families tested		
	Active	**Non-Active**	**Total**	**Active**	**Non-Active**	**Total**	**Active**	**Non-Active**	**Total**
***P. tecunumanii* LE**	3	17	20	6	12	18	66	260	326
***P. tecunumanii* HE**	1	26	35	3	19	22	37	371	408



9 & 10 year volume/tree (m^3)

	Altitude	MAP
Windy Hill	930 m	1039 mm
Grootgeluk	1120 m	940 mm

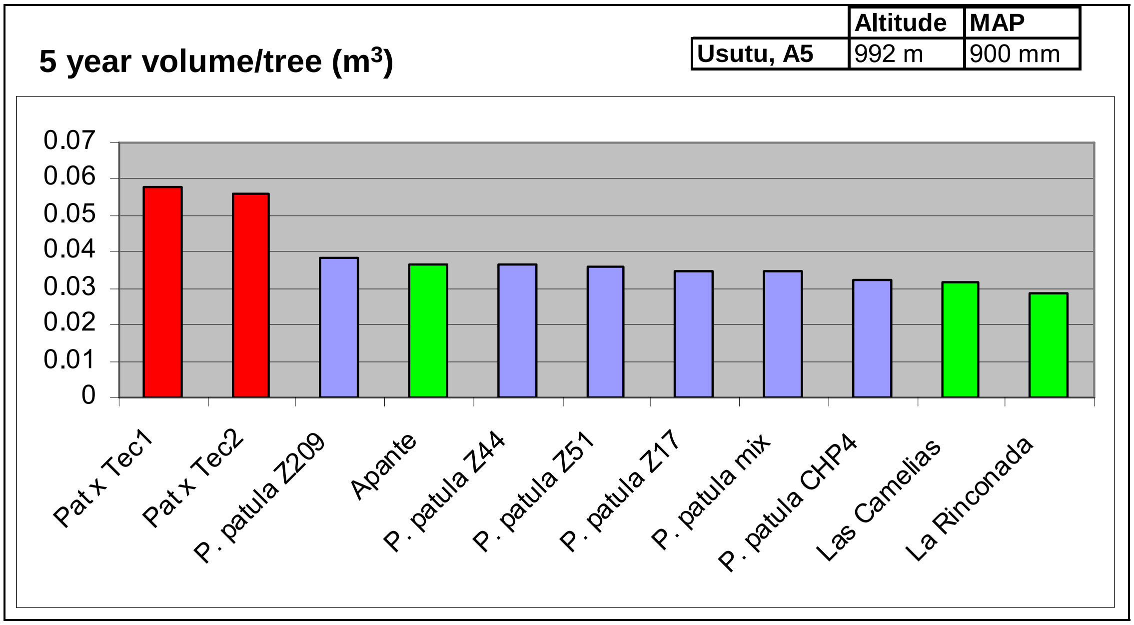
Results are presented on volume growth, adaptability and cone production from a range of OFI and Camcore trials planted in South Africa and Swaziland. A number of different provenances of *P. tecunumanii* perform well under South African conditions (Fig 1 and 2). Site A in Fig 2 is at an altitude of 820 m and site C at 1241 m a.s.l. Results from these trials have been used to select plus trees from well adapted provenances. Second (2^{nd}) generation selections were established in a progeny trial series in 2004 to facilitate further genetic improvement. A wide range of genotypes and provenances, many with vulnerable to endangered conservation status in their natural ranges, have also been conserved in the original trials, clone banks and clonal seed orchards.



7 year volume/tree (m^3)

Results from the trial series' have also aided in better matching of species to site to optimize the species' productivity as well as flowering and seed production in clone banks and clonal seed orchards.

Very good flowering has been found for high elevation *P. tecunumanii* provenances in clone banks established at an altitude of around 1300 m. Low elevation provenances flower better at an altitude of around 1000 m. Currently, *P. tecunumanii* shows much commercial promise as a hybrid partner with *P.patula* and has shown improved tolerance to the pitch canker fungus in seedling and field inoculation trials.



The first controlled crosses of *P. patula x P. tecunumanii* were carried out in 1992. Several studies relating to controlled pollination, flower and pollen morphology have also been completed and have further broadened the knowledge of the species. To date, a total of 25 field trials and block plantings have been planted testing the *P. patula x tecunumanii* hybrid and results are promising (Fig 3).

Acknowledgements: The use of data from various data reports produced by Camcore and Sappi is gratefully acknowledged. Assistance provided by William Woodbridge of Camcore with the compilation of some of the tables is also acknowledged.

Keywords: selection, provenance, hybrids, controlled pollination, *Pinus tecunumanii*

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Morphological Differentiation And Hybridization Between *Quercus alnifolia* Poech And *Quercus coccifera* L. (Fagaceae) In Cyprus

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Abstract

Analysis of morphological traits was carried out in order to provide insights regarding differentiation and hybridization between two evergreen oak species, the golden oak (*Quercus alnifolia* Poech) and the holly oak (*Quercus coccifera* L.) in Cyprus. The holly oak shows a higher degree of morphological diversity, in comparison to the endemic golden oak, which is confined to the ultrabasic rock formations of the Troodos Massif. The parental species can be clearly distinguished. Each species forms both pure and sympatric populations; no significand differences were observed at the within species level. Analysis of mixed stands indicates active but limited genetic introgression and hybridization between the two oaks. Designated hybrids form a distinct group in multivariate space being morphologically closer to the golden oak. This may indicate a maternal effect and provides some information about reproductive patterns. Reclassification of some putative hybrids within parental groups and vice versa can be attributed to backcrossing events. The present study is the first regarding hybridization of the above species and could serve in characterising the two oak species and identifying their hybrids. A further study based on genetic markers focusing on reproductive patterns, genetic diversity and private alleles would be highly interesting.

Keywords: Genetic introgression, ecological barriers, backcrossing, maternal effect

Growth and Stem Quality Variation In Irish Birch

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Abstract

Two species of birch are native to Ireland; *Betula pubescens* Ehrh (downy birch) and *Betula pendula* Roth (silver birch). Two factors have prevented their listing as a recommended species; poor stem quality of native birch and poor survival and growth rates of imported birch. Both species have been included in an improvement programme based on native material. Provenance and progeny trials were established at two locations with the two species planted adjacent to each other. Height, diameter at breast height (DBH) and stem quality were assessed after four growing seasons. The data for different groups (species/provenance/progeny) has been analysed separately. There were significant differences in the groups for the traits assessed. For example, ANOVA analysis of height values revealed significant differences between seed source for sixteen *B. pendula* provenances ($F = 9.703$; $df_{15, 676}$; $P \leq 0.001$) and for twentyfive *B. pubescens* provenances ($F = 4.829$; $df_{24, 1050}$; $P \leq 0.001$) studied. The site*provenance interactions were non-significant and the ranking of the provenances was similar for each location. Heritability estimates for growth characters were high; family heritability (h^2_{ns}) estimates of 0.825 for height and of 0.841 for diameter for eight open-pollinated *B. pendula* families and 0.846 for height and of 0.861 for diameter for nineteen full-sib *B. pubescens* families. Coefficients of variation within the *B. pubescens* crosses ranged from 0.142 to 0.264 for height and from 0.302 to 0.556 for DBH. Many stem defects had already become apparent. Overall, only ~30% of trees were classified as having good stem form. However, stem form was associated with provenance (or family) e.g. $\chi 2 = 50.99$ (36 df, $P < 0.05$) for *B. pubescens* provenances. The results indicate that there is a good basis to improve growth rates, at least in the early growth phase, but that selection for stem quality will reduce the breeding population and slow progress.

Key words: *Betula pendula*, *Betula pubescens*, provenance, progeny

Identification of Turkish Sweetgum (*Liquidambar orientalis)* Varieties by Studying *Trn* Regions of Choloroplast DNA

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Abstract

Liquidambar genus are represented with five species in the world and one of these species, Turkish sweetgum *(Liquidambar orientalis)* is naturally found only in southwestern Turkey with limited distribution in Muğla Province. Apart from being a relic endemic specie of Turkey, its limited distribution with three recognized varieties and the presence of increasing threats to its genetic resources signify the importance of studying genetic diversity in this specie. This type of information could be used to plan better conservation and management programs for *L.orientalis*. For this purpose, 18 different populations were sampled throughout the species range and noncoding tRNA region, *trn* region, of chloroplast DNA is being studied to asses the genetic variance within the species. Experimental studies included the extraction and quantification of cpDNA, amplification of the *trn* region by PCR reaction using two sets of primers and sequencing of the region to detect the genetic variance. Sequencing was performed by dye-termination method and the collected sequence data were used to conduct phylogenetic and molecular evolutionary analyses by using MEGA version 3.1 (Kumar, Tamura, Nei 2004). The data collected so far indicate that *trn* region shows significant intragenic differences for *L. orientalis* because of the high mutation rates of the region. Results from this study shows that this region is a suitable tool for studies dealing within species diversity. The distances within groups composed of three different varieties (*unknown, integriloba* and *orientalis*) are found to be 0.040, 0.044 and 0.023 for *unknown, integriloba,* and *orientalis,* respectively. When the distances are computed between groups, they were 0.042 between *unknown* and *integriloba*, 0.032 between *orientalis* and *integriloba*, and 0.029 between *integriloba* and *orientalis*. The mean diversity between varieties is found to be 0.036. Likewise the mean diversity for all populations is 0.040 and the coefficient of differentiation is 0.095. From the phylogenetic tree constructed based on the preliminary results of the study, it was seen that the variety *integriloba* is only one that grouped distantly from the other varieties. When the study is completed with additional individuals per population and *trn* region, the results will be more meaningful and allow firm conclusions.

Key Words: *Liquidambar orientalis*, trn, cpDNA, genetic variance

Genetic Structure of Turkish Sweetgum (*Liquidambar orientalis)* Populations and Identification Varieties with the Study of *matK* Region of cpDNA

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Abstract

Liquidambar genus are represented with 5 species in the world and one of these species*,* Turkish sweetgum *(Liquidambar orientalis)* is naturally found in only soutwestern Turkey, mainly in Muğla Province. Turkish sweetgum is a relict endemic to Turkey. The limited distribution of species with three recognized varieties and increased threats to its genetic resources signify the importance of studying genetic diversity in the species to have better conservation and management of genetic resources. For this purpose, 18 different populations were sampled throughout the species range and *matK* region of chloroplast DNA was studied to asses the genetic structure of the species. *MatK* region was chosen for analysis because many studies have documented the utility of this gene for resolving phylogenetic relationships at a variety of taxonomic levels, from closely related species to the family level(e.g., Johnson and Soltis, 1994, 1995; Soltis *et al*., 1996; Hilu and Liang, 1997)The extraction and quantification of cpDNA were completed for all 18 populations. Eight pairs of primers were designed to amplify the *matK* region and PCR products were digested with 5 restriction enzymes (*MboI, BsaBI, TaqI, AluI, XagI*) to reveal the polymorphism in sampled populations. However, these restriction enzymes did not produced polymorphic fragments that could be used for studying polymorphism in Turkish sweet gum. Selected 4 *matK* primers and 1 individual from each of 14 populations were sequenced. Sequence alignments were made visually. Phylogenetic analysis were conducted with these sequences, using the software MEGA3.1 (Kumar, S., K. Tamura, and M. Nei. 2004). The aligned *matK* sequences were 1512bp in length. Among three varieties of *L.orientalis* namely *orientalis*, *integriloba* and , *unknown*; *integriloba* and *unknown* seem to be genetically closer (genetic distance:0.66) than *orientalis* and *integriloba* do. Distance between *orientalis* and *unknown* is quite large (genetic distance:0.72). The constructed phylogenetic tree among the populations of these three groups consist of 5 clusters including 2 major groups (2 *integriloba* and 3 *unknown* populations). Clustering of varieties in strict consensus tree was not related to results of early taxonomic studies. This study is a preliminary genetic study to discriminate oriental sweet gum varieties in Turkey. Therefore, the research will be continued by using more primers for *MatK* region and including more individuals per population.

Induction of Embryogenic Tissues from Immature Embryos in *Pinus nigra* subsp. *pallasiana* Lamb.

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Abstract

Several economically important tree species belong to the genus *Pinus.* Pine wood is a major raw material for the forest industry. Because of the importance of some pine species, advances in micropropagation especially somatic embryogenesis is essential for clonal forestry. Anatolian black pine (*Pinus nigra* subsp. *pallasiana*) is one of the important timber species and used widely in afforesting the high Anatolian steppes. To propagate the species clonally, embryogenic tissue initiation (ET) was obtained using immature zygotic embryos. The experiments were carried out in the years 2004 and 2005 by collecting immature cones weekly (7 weeks) from June to August. DCR (Douglas-fir cotyledon revised) medium, supplemented with 13.6µM 2,4-D (2,4-dichlorophenoxyacetic acid), 2.2µM BAP (6-benzylaminopurine), 0.5 g/L casein-hydrolysate, 0.25 g/L L-glutamine, 3% sucrose and 0.2% gelrite was used for initiation. ET initiation frequencies were calculated for each family/collection/year combinations and analyzed. Overall initiation frequencies for collection dates were calculated as 0.89% for 2004 and 1.92% for 2005. Among the cone collection dates, the highest initiation frequency was recorded for July-5 2005 (3.94%). Also, significant differences between trees as well as interaction between collection dates and trees were found.

Keywords: *Pinus nigra* subsp. *pallasiana,* micropropagation, somatic embryogenesis, initiation, Douglas-fir cotyledon revised medium.

The Effect of Gibberelline $A_{4/7/9}$ ($GA_{4/7/9}$) Injection on Flower Production in a Turkish Red Pine (*Pinus brutia* Ten.) Seed Orchard

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Abstract

Turkish red pine (*Pinus brutia* Ten.) is one of the important tree species in National Tree Breeding Program in Turkey. Totally 2170 plus trees have been selected from the natural stands and 67 seed orchards covering 460 ha. have been established. The main goal in seed orchard management is to reduce seed cost while increasing physiological and genetic quality of the seed crop both of which is dependent on flower production. In order to increase flowering in the seed orchards, stem injection of gibberelline $A_{4/7/9}$ ($GA_{4/7/9}$), banding with wire, $GA_{4/7/9}$ injection combined with banding with wire were applied in a Turkish red pine seed orchard established with 30 clones in 1992. Ten out of 10 clones in the orchards were subjected to the study. Banding with wire was started in early spring and ended with the first stem injection. Each treated tree with injection of $GA_{4/7/9}$ was received 2.5 mg of $GA_{4/7/9}$ in the first and second half of July in years 2001-2003. Male and female flowers were counted and the distribution of flowers on crown was explored. The treatments did not cause any destructive effect on trees health, height and stem diameter growth. Treatments did not affect female flower production, however, both $GA_{4/7/9}$ injection and $GA_{4/7/9}$ + banding with wire treatments lead to 2-3.5-times increase in male flower production compared to untreated trees. Poor male flowering clones responded to $GA_{4/7/9}$ treatments much more than good flowering clones.

Key words: stem injection, banding with wire, flower stimulation

Clonal Propagation and ex situ Conservation of the Ideotype *Picea abies pendula* through Biotechnological Methods

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Abstract

The aim of this paper is to present recent results of the researches concerning the use of advanced biotechnological methods (propagation by somatic embryogenesis, ex situ conservation through tissue cultures) in connection with the breeding program of narrow crowned Norway spruce. The somatic embryogenesis was induced out of cotyledons of seedlings, buds and needles. Several somaclones were isolated and kept for years, as "living collection". Various methods for the conservation of embryogenic somaclones were tested. The most successful were the encapsulation and cold preservation of the embryogenic masses, and the cryopreservation of PEM. In order to perform the cryopreservation without controlled prefreezing, we simulated the program of the cryostat by immersion of cryoprotected tissue for one hour in alcohols cooled at -20oC. The preservation of regeneration ability in recovered embryogenic tissues and embryos, prevention of senescence and possible genetic drift were analyzed.

Keywords: Picea abies pendula, somatic embryogenesis, clonal propagation, conservation, encapsulation, cryopreservation

Introduction

The narrow-crowned Norway spruce (Picea abies pendula) is an ideotype characterized by higher resistance to wind and snow damages. The genetic background of this ideotype seems to be a mutation placed of a dominant gene. The evidence of the pendula character occurs only after the age of 20-25 years. The absence of a marker for the early evidence of the pendula traits make necessary the conservation of regenerative breeding material, till the ideotype will be expressed. The aim of this paper is to present the recent results of the researches concerning the use of advanced biotechnological methods (propagation by somatic embryogenesis, ex situ conservation through tissue cultures) in connection with the breeding program of narrow crowned Norway spruce.

Material and Methods

The somatic embryogenesis was induced out of various explant types, including cotyledons of seedlings, as well as non-juvenile explants (buds and needles). Various culture media were tested, all based on GD nutrients (Gupta and Durzan, 1986), containing auxins and cytokinins in different proportions (Palada-Nicolau, 1999). The observations were made in two steps (the apparition of first embryos and the formation of embryogenic masses - PEM) and the efficiency of embryo induction, respectively stabilization, was evaluated. The stable embryogenic cultures were managed into a synchronous embryogenic system, based on 5 stages of embryo development, each stage being initiated by a special composition of growth regulators in the culture medium: auxins and cytokinins for embryo induction and proliferation of PEM, abscisic acid for embryo maturation, hormone-free medium containing active charcoal for germination and conversion.

Numerous somaclones were isolated and kept for years, as "living collection". Meantime, the somatic embryos were synchronously matured and emblings were regenerated. The structural quality of the somaclones maintained on GDX medium was analyzed according to an original method (Palada-Nicolau, 1999). Various methods for the conservation of embryogenic somaclones were tested. The encapsulation of PEM in calcium-alginate was tested as a practical method for the medium-time conservation (for several months) of somaclones at positive temperatures. The cryopreservation of Norway spruce ESM without controlled freezing was performed according the method of Hargreaves and Smith, 1994, with modifications. Various combinations of freezing solutions and cryoprotective agents were tested. The results were compared with them obtained in the classical cryopreservation technique using controlled freezing (Kartha et al., 1988)

Results and disscussion

The induction of somatic embryos out of juvenile explants

Using culture media previously optimized (providing 90-100% induction efficiency out of emblings), 20 narrow crowned Norway spruce maternal progenies from two locations were tested. The efficiency of embryo induction ranged between 3.92% and 34.16%, in the case of mature embryos and between 15% and 32.96% in the case of cotyledons (mean of three replications), with maximal values over 65% in embryos and 57% in cotyledons. The distribution of the values of embryo induction efficiency found in the two locations (Remeti and Fancel) are shown in the figures 1 and 2.

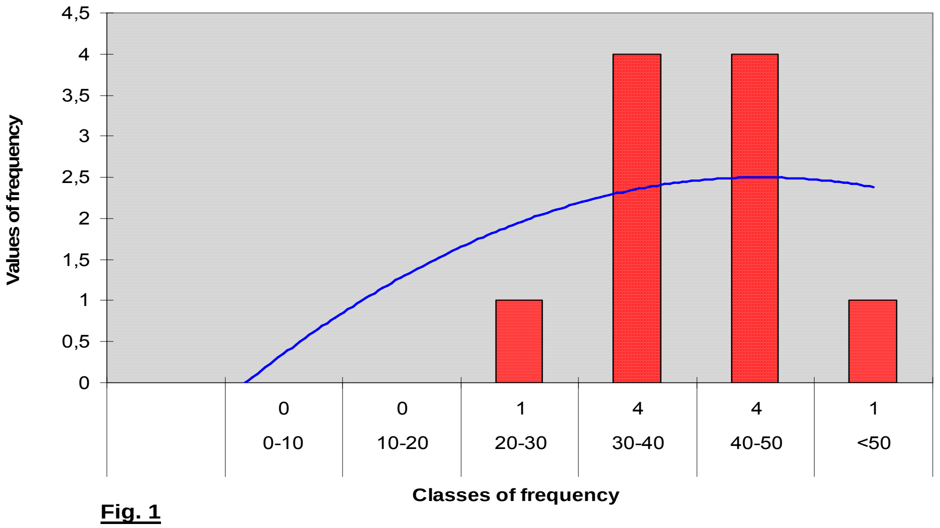


Fig. 1
The distribution of the frequency of embryo induction efficiency in 10 narrow-crowned Norway spruce maternal progenies from Stana-de-Vale (O.S.Remeti)

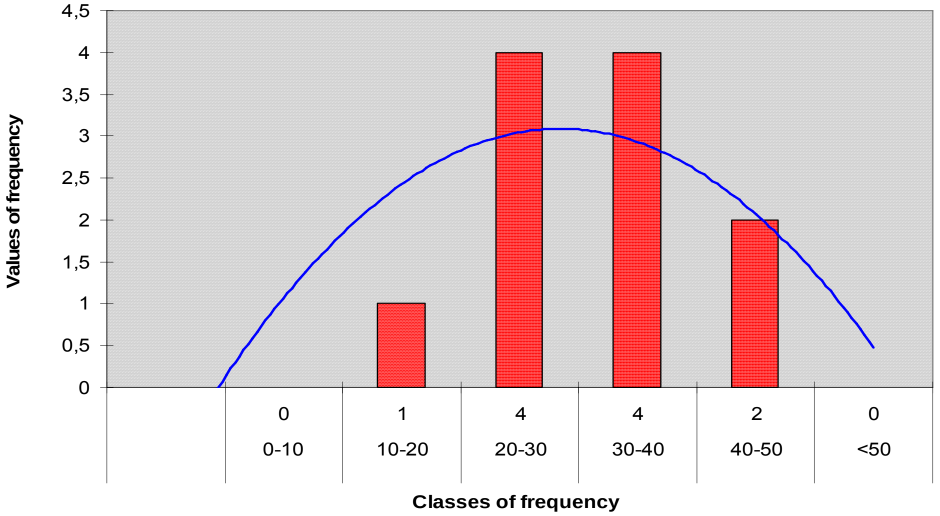
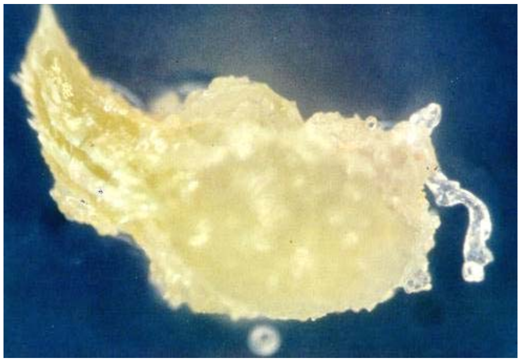


Fig. 2
The distribution of the frequency of embryo induction efficiency in 10 narrow-crowned Norway spruce maternal progenies from the population Targu-Mures (O.S.Fancel)

The induction of somatic embryos out of non-juvenile explants (winter buds and needles)

The experiments presented here are directed to the extension of the available embryogenic explants toward the less juvenile ones (needles, buds), in order to make possible the clonal propagation of selected trees, especially of those belonging to the narrow-crowned ideotype *Picea abies f. pendula*. The embryo induction and the stabilization of embryogenic somaclones was possible out of needles selected in a suitable developmental phase from activated buds (Fig. 3 na 4)



The efficiency of embryo induction out of the non-juvenile explants was generally low, comparing with that of juvenile explants, ranging between 5 and 11,4 % (Fig. 5).

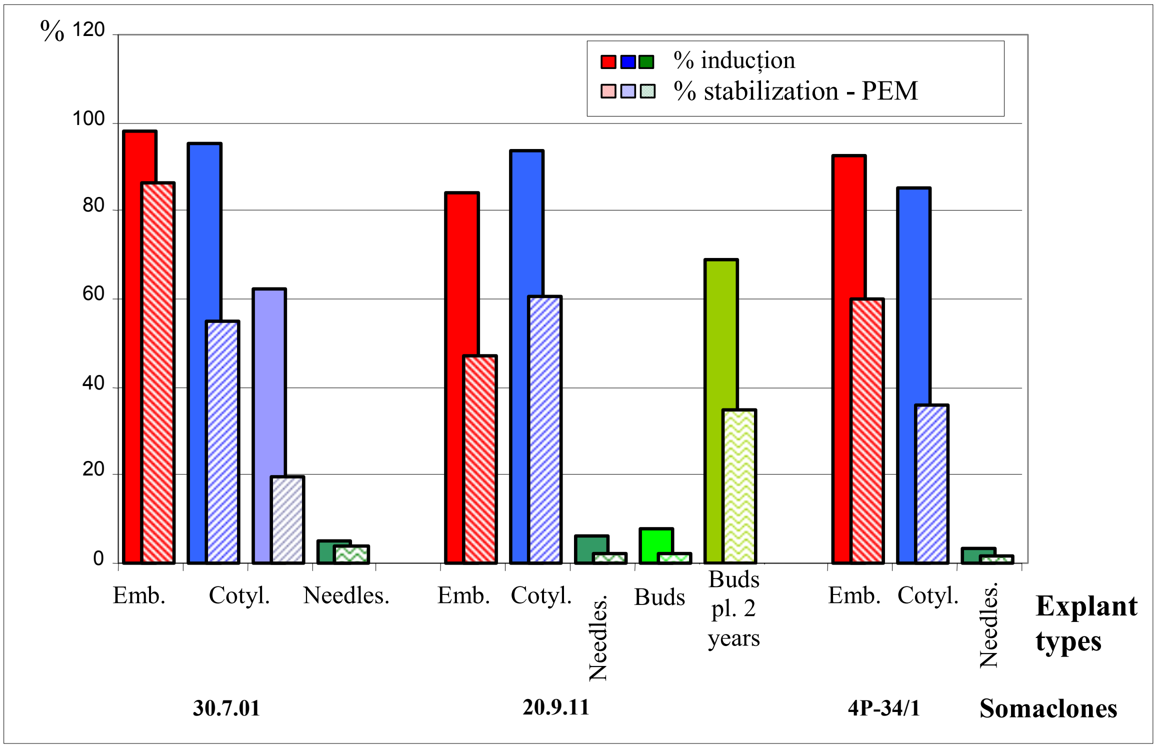


Fig. 5 - The effect of explant type upon the efficiency of somatic embryo induction at three somaclones of common and narrow-crowned Norway spruce

Only in one case (two-years old emblings of one somaclone), the embryo induction efficiency out of needles was comparable with that of cotyledons. (Fig. 6)

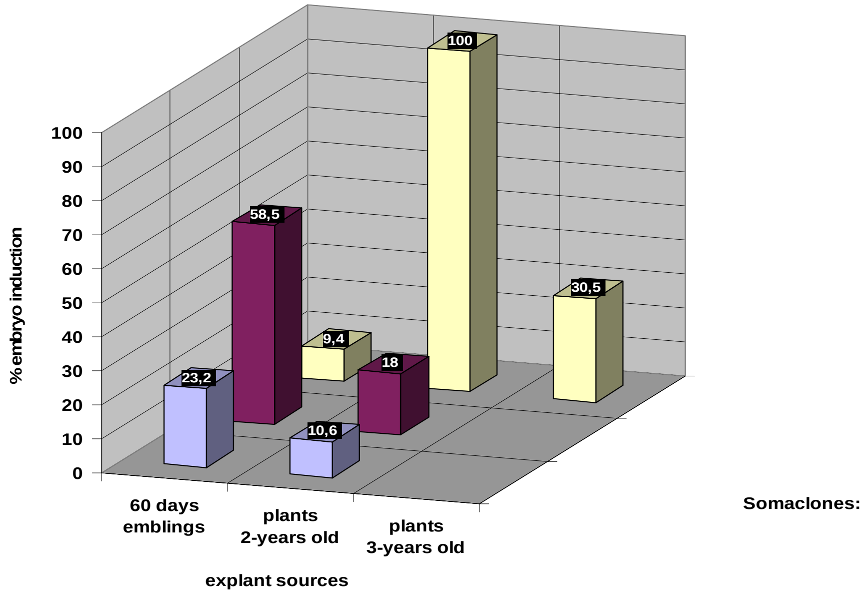


Fig. 6 - The efficiency of somatic embryo induction out of non-juvenile explants (buds, needles)

The stabilization and long-term culture of the embryogenic lines isolated from the PEM obtained out of needle explants was carried out in the same way as in the case of cotyledon originated PEM and with similar results. The efficiency of embryo maturation and germination and the quality of mature embryos and emblings were also comparable with those of juvenile explant originated somaclones. Normal, acclimatable plantlets were regenerated, as well as from juvenile explants.

PEM stabilization and selection of embryogenic somaclones

Long-term culture and living collection

The Norway spruce (*Picea abies f. pendula*) embryogenic somaclones of monoembryonal origin, isolated from primary induced embryogenic tissue, were maintained for several years in long-term culture, as embryogenic masses (PEM) regularly subcultured on proliferation medium GDX. The senescence of embryogenic lines, expressed by the decreasing of embryo maturation efficiency, degradation of structural quality and increasing of terratogenesis, occurred usually after two to four years. The age effect (senescence) upon the structural quality of somaclones and upon the maturation efficiency of three narrow-crowned Norway spruce embryogenic lines (4P-34/1, 4P-34/2 and 4P-34/7) are illustrated in the figures 7 and 8. In order to increase the duration of the preservation of embryogenic lines as living collection (an alternative way to cryopreservation), the embryo reinduction was carried out, using mature somatic embryos of early senescent lines. The reinduction offered the possibility of recovering the good qualities of mature, fully established embryogenic lines for a couple of years, (fig. 9 and 10.). The behaviour of three primary embryogenic somaclones and that of their reinduction “progenies” was analised.

Unexpected variation in the behaviour of the “sister-lines” (primary, as well as secondary and tertiary reinduction lines) occured, in terms of:

- polymorphic behaviour of early isolated somaclones, reflected in some difficulties of stabilisation;
- stabilisation ability (more the original line is older, more the necessary time for the stabilisation of secondary reinduced lines is shorter)
- major differences between “sister-lines” of the same reinduction series, concerning maturation efficiency
- variation between “sister-lines” of the same reinduction series, concerning even the shape and dimension of mature embryos, characters usually stable and definitories for the embryogenic lines of the same origin.

These variation, expressed in the primar somaclones and their secondary and tertiary “somatic progeny” is shown in the figures 9 and 10.

The embryogenic lines used in this study being true somaclones, isolated by the culture of isolated globular embryos, and moreover, the reinduction being carried out also with single embryos (the process of isolation of somaclones with monoembryonal origin repeated), it is rather hard to explain this variation in the behaviour of “sister-lines”

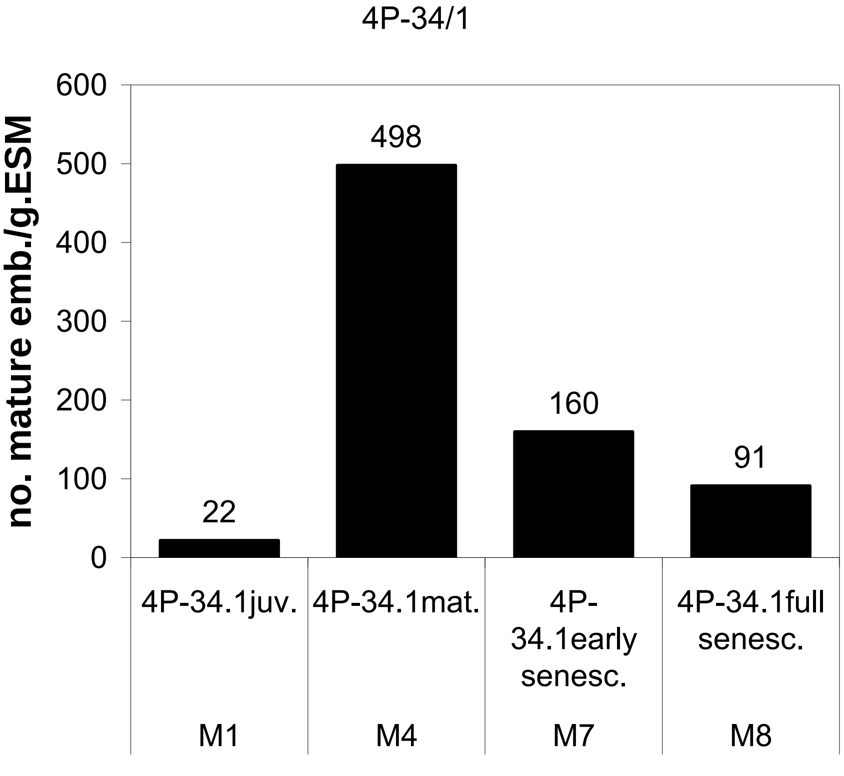


Fig. 7 - The age effect (senescence) upon the structural quality of somaclones

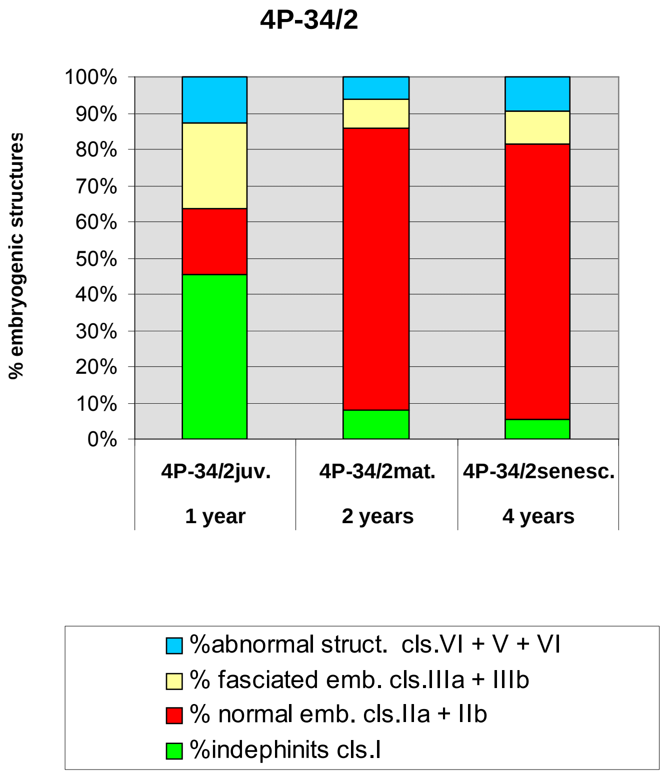


Fig. 8 - The age effect (senescence) upon upon the maturation efficiency

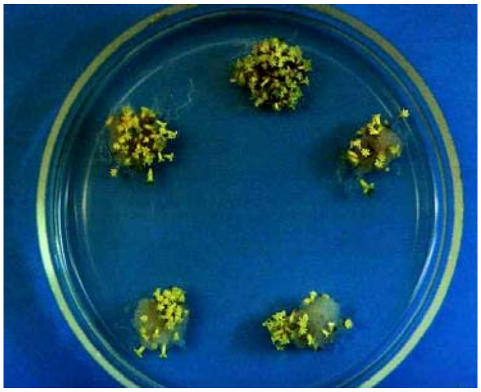


Fig. 9 - Senescent primary line

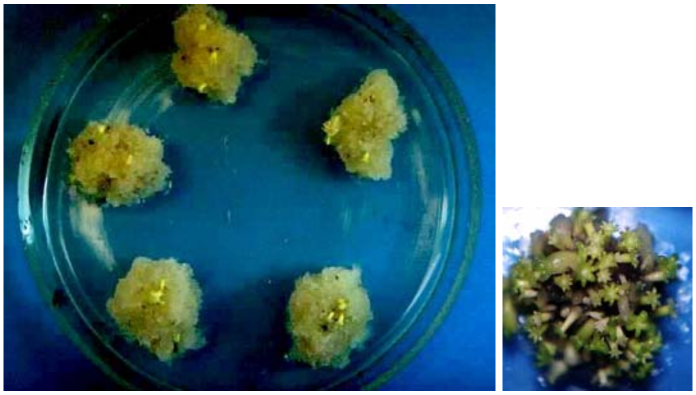


Fig. 10 - New generation reinduction lines

Synchronous maturation, germination and plant regeneration

The regeneration of plants (emblings) was carried out in a synchronous way, by the transfer of a part of the PEM on suitable media for the progress toward a more advanced stage of embryo development, as is shown in the figure 11.

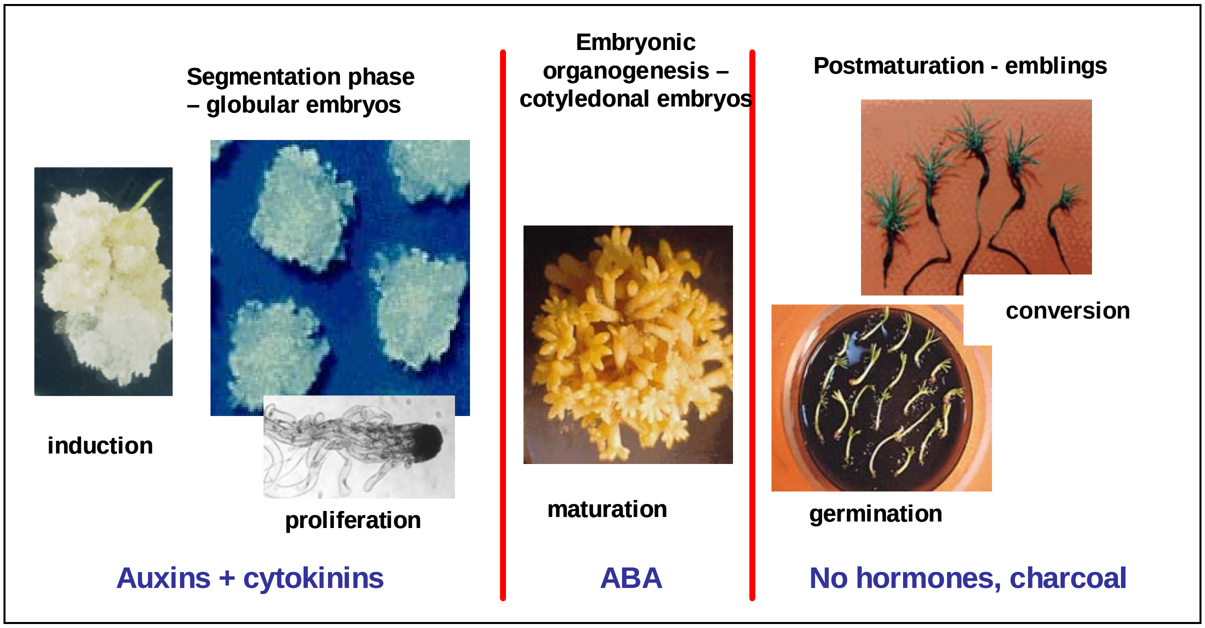


Fig. 11 - Illustration of the basic method of plant regeneration of Norway spruce by somatic embryogenesis, based on the results of the author

Conservation of embryogenic somaclones by encapsulation

The method of encapsulation in calcium-alginate, designed for the preparation of artificial seeds, was improved and successfully used for the medium-term conservation of narrow-crowned Norway spruce embryogenic somaclones, as PEM. The PEM from a dense suspension, previously cultivated in GDXL medium (Fig. 12), were embedded in calcium-alginate beads (Fig. 13 a), placed in Petri dishes with a drop of sterile distilled water, sealed and conserved for periods from 7 days to two months at 4°C, in refrigerator. The procedure was considered successful when the embryogenic cultures restarted the growth after several days of culture at 22 °C. (Fig. 13 b-f.). As conditions for encapsulation, different variants for the density of suspension and the number of days from the last subculture were tested. The best results were obtained with dense suspensions (100-200 mg ESM / ml medium) harvested after 7 days from the last subculture on GDXL medium.Tests of viability (the culture of 25 conserved beads in a Petri dish with solidified GDX medium) were made after 7, 30 and 60 days of conservation. The results are shown in the fig. 14.

Cryopreservation of narrow-crowned Norway spruce embryogenic somaclones

In order to perform the cryopreservation without controlled prefreezing, we tried to simulate the program of the cryostat by immersion of cryoprotected tissue for one hour in alcohols cooled at -20°C. The main aim of this experiment was the establishment of a simple and cheap cryopreservation method that could work without any special, unaffordable equipment, as a cryostat. Using a plastic box filled with an alchool (as stabiliser) and a home freezer, we have realized a freezing program that can simulate exactly that of a cryostat, freezing the cryoprotected material in one hour from +5 to –15,5°C. In order to complete thr pre-freezing program to -30 °C, the material was exposed for 5 min. to the nitrogene atmosphere in the cryoconservation bottle, above the level of the liquid nitrogen, before the complete imerssion

(Fig. 15). Comparisons between cryopreservation (with and without controlled freezing) and encapsulation were made, and the genetical implications of each procedure were analyzed. The long-term culture of embryogenic lines (living collection) is considered as reference for the physiological status of cultures and for the conformity of regenerated plants. The preservation of regeneration ability in recovered embryogenic tissues and embryos, prevention of senescence and possible genetic drift were analyzed.

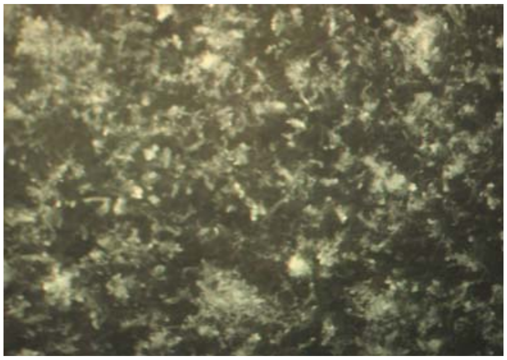


Fig. 12 – Suspension of Norway spruce PEM in GDX medium containing Na-alginate, before encapsulation

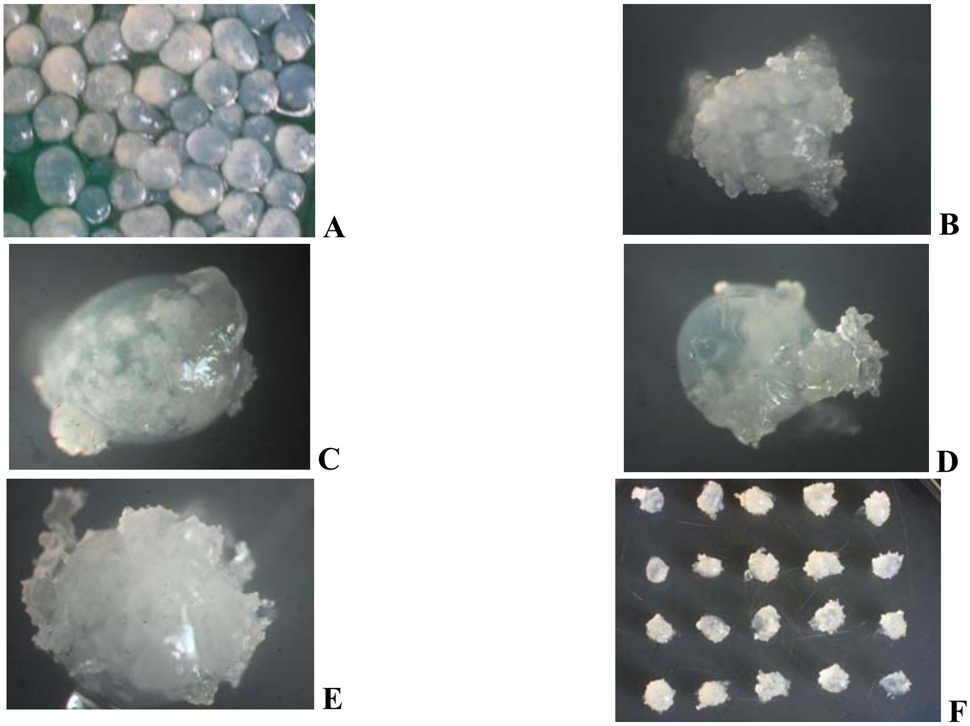


Fig. 13 – The recovery of embryogenic masses after conservation as alginate beads
a –alginate beads containing PEM; prepared for conservation; b – proliferation inside the bead; c – străpungerea capsulei şi ieşirea primilor embrioni; d – multiplicare extracapsulară; e – formarea ESM; f – imagine de ansamblu ilustrând randamentul mare al reconstituirii

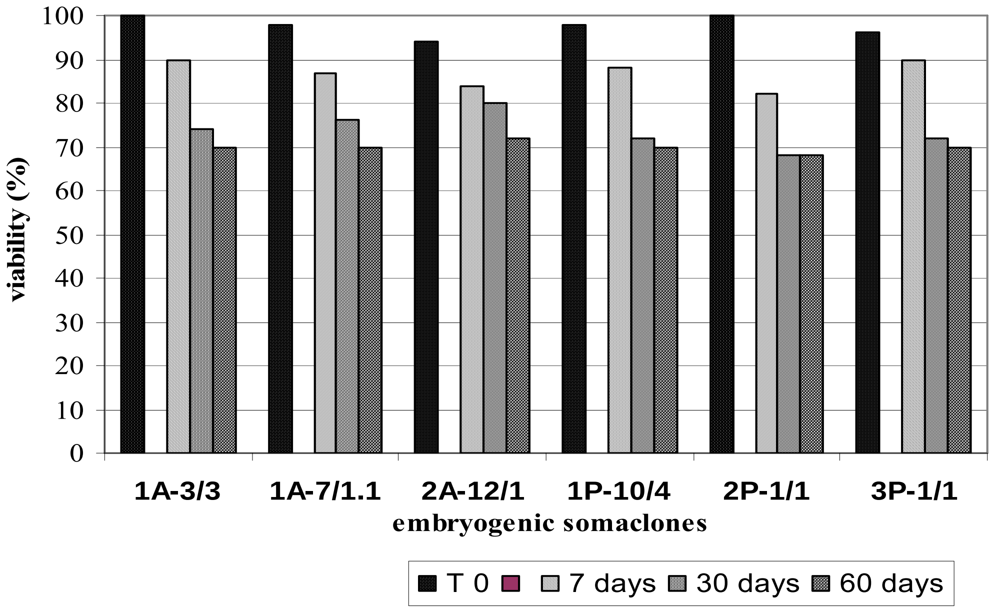
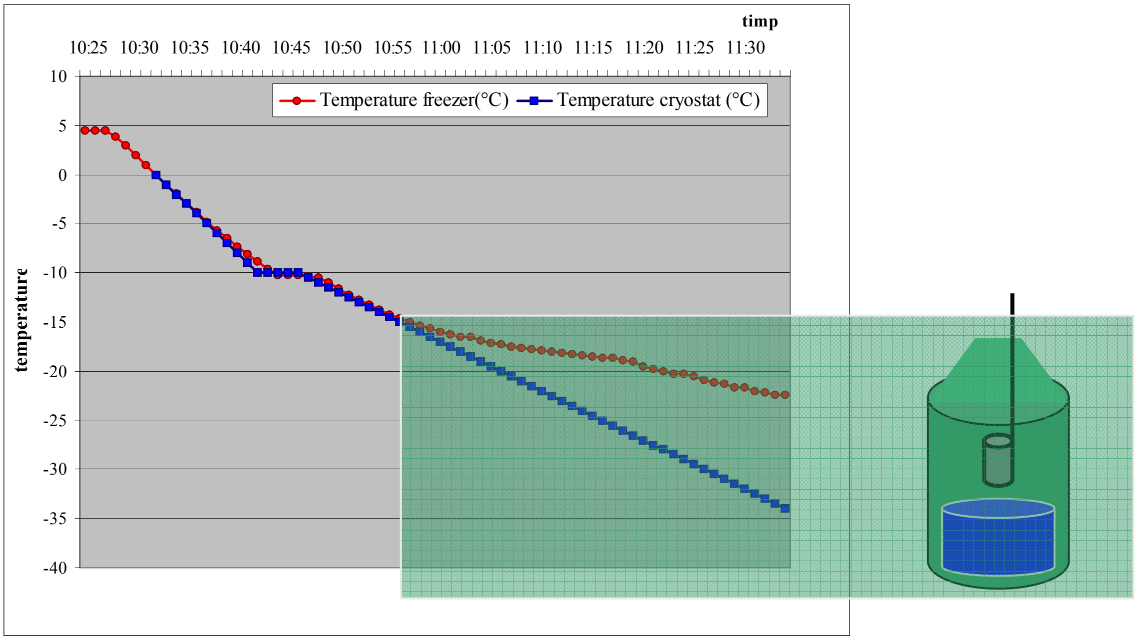


Fig. 14 - **The effect of the duration of conservation of PEM as calcium-alginate beads upon the viability of embryogenic cells**



Conclusion

- The somatic embryogenesis was induced out of various explant types, including cotyledons of seedlings, as well as non-juvenile explants (buds and needles).
- Several somaclones were isolated and kept for years, as “living collection”. Meantime, the somatic embryos were synchronously matured and emblings were regenerated.Two alternative methods for the conservation of embryogenic masses (ESM) offered the possibility for storing the somaclones of our collection: encapsulation in Ca-alginate and cryopreservation.
- The controlled pre-freezing was simulated in a simple home freezer. The efficiency of recovering in such conditions was comparable to that of controlled pre-freezing in a cryostat.

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Hybridization Strategies on Gene Conservation of Endangered Sandalwood

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Abstract

Previous study on the breeding system of *Santalum album* (Sandalwood), an endangered tropical trees planted on ex-situ gene conservation in Wanagama, Central Java, indicated the mixed mating and self-compatibility mechanism that resulted on the highly selfing-rate. Pollen limitation brought to the low seed set, particularly on the natural pollinated fruit. Increasing pollen quantity and intensity seemed to have significant effects on pollination effectiveness and seed set. Cross-pollination resulted on highest seed set compared to those of self and open pollination. This research was aimed to increase the pollination effectiveness and crossing rate of this rare species by hybridized 3 provenances of Karangmojo (K), Tilomar (T) and Imogiri (Im). Result observed the high Pollination Effectiveness on the each of cross-hand pollination of T, Im and K (35; 27; and 25%) compared to its natural pollination (2.1; 1.7 and 1.7), respectively. High seed set also observed on cross-pollination of K, T, and Im (21.9; 15.3; and 12.6%) compared to its natural pollination (0.96; 0.89 and 0.93), respectively. Combination of T (female) and K (male) resulted on the highest rate of Pollination Effectiveness (64.88%). The combination of T and K; both as male and female parent; resulted on highest seed set. Seed weight and size were affected by female parent: K pollinated flowers always produce the most weight seed; while that of T always produce the biggest seed. Time of seed maturity was strongly affected by male parent. Flowers pollinated by K pollen always mature longer than those pollinated by Im pollen. It was recommended to hybridize T and K; both as male and female parent; to achieve the high seed productivity. Focus on the next research should be made on estimating the genetic gain achieved by hybridization.

Keywords: Santalum album, hybrid, provenances, pollination effectiveness, crossing rate, seed set, seed weight, seed size

F_1 hybrid pollination trials on *Eucalyptus globulus* and *Eucalyptus camaldulensis* in the semiarid region of Chile

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Abstract

Eucalyptus globulus is the most important exotic species for pulp and paper production in Chile due to exceptional chemical and physical wood properties. In the best sites (costal and mild temperate zones of the south of Chile) with intensive silviculture technology and improved genetic material (controlled crosses seed, elite's clones propagated by cuttings) the species can achieve more than 40 m3/ha/year. Nevertheless there are some limitations to extend commercial plantations for other zones in Chile. Frost damage is the main problem for planting in the south and a broad dry season in the north and interior valleys (called "secano"). INFOR has developed a large tree breeding program that included species / provenance / progeny trials, o.p. family's selections and elite clones for different soil and climatic conditions.

Three"*base species*" has been selected: *E. camaldulensis*, *E. cladocalyx* and *E. sideroxylon* for the semiarid region of Chile with an annual rainfall less than 200 mm which show good adaptation to drought and soil conditions but have one or more restrictions for industrial and commercial uses like : (a) slow growth (4 - 7 m3/ha/year), (b) deficient form of the trees and (c) low quality of wood properties. Controlled cross matrices were designed using one stop pollination technique to improve one or more of these traits. Pollen corresponds to "*improved species*" like *E. globulus and E. camaldulensis*. Capsule survival, seeds/capsule, seed/flower pollinated and rooting were measured by hybrid progeny.

This paper show preliminary results obtained after two pollination seasons of hybrid seedlings and clones obtained using O.S.P. technique on *E. globulus* and *E. camaldulensis* females. This genetic material will be planted on progeny and clonal trials for operational production using mother "stock plants".

Keywords: hybrid, O.S.P., rooting ability, pollination.

Mediterranean Cypres (*Cupressus sempervirens* var. horizontalis) **Provenance-Progeny Trials: An ex-situ conservation**

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Abstract

Provenace-progeny trials were established at three sites using seedling sampled from 16 natural populations in Mediterranean and Aegean Region in Turkey and 1 from Samos Island in Greece. Sample sized varied between 2-68 families/population. Height and diameter at collar were measured for early study test end of the first growing seasons in Denizli forest nursery. Highly significant differences ($Pr<0.001$) were observed between and within population for height and collar diameter at age one.Variation observed among populatation was 18 and 17 % for height and diameter respectively, however, within popuation variation was 21and 9 % respectively. Individual heritebility (h_i^2) values estimated for height and diameter at collar was 0.25 and 0.11 respectively. In addition, family heritability (h_f^2) value obtained was 0.89 and 0.77 for height and diameter at collar characters. Family heritability values were higher than the individual heritability values for both characters studied. Repeatabilty value for height and collar at diameter was observed 0.73 and 0.84. Among population and within population variation indicate that this tree species appropriate for breeding study for genetic gain. Mediterranean cypress natural popuations are vulnerable to forest fire, over grazing and clear cutting. For this reasons, natural cypress populations must be consrvation by both *ex-situ* and *in-situ* programs.

Keywords: Mediterranean Cypress, hight, diameter at collar, genetic variation, heritabily, repeatabilty

Inheritance Level of Leaf Reverse Color in *Acer pseudoplatanues* L. cv«*Atropurpureum*»

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Abstract

Acer pseudoplatanus L. is one of the most important hardwood tree species in Serbia. This deciduous tree is very well adapted on urban areas conditions and it is used often for horticultural purposes. *Cv «Atropurpureum»* is characterized with very interesting decorative trait, red colored leaf reverse, which contributes to red color of stems crowns. With the aim of inheritance level estimation of mentioned trait in F1 generation, the selection of mother trees and seed collecting have been organized. Offspring leaf reverse color has been analyzed and average inheritance of leaf reverse red color has been estimated as 50%. Mother genotypes, with offspring characterized with percent of analyzed trait inheritance higher than 50%, should be included in further breeding program processes, with the aim of improved decorative seedling material production.
Keywords: Sycamore, decorative trait, breeding program

Introduction

The history of ornamental tree culture is one of the classical examples of the plant modification under the influence of the environment or breeding, which confirms undoubtedly that plant breeding is the evolution process directed by man. The reasonable and planned reconstruction of the actual tree species and the construction of the new cultural forms (cultivars) with a more or less familiar genetic constitution, for the needs of urban forestry, imposes the following tasks to tree breeders: the increase i.e. decrease of productivity, enhancement of aesthetical characteristics, increase of resistance to diseases, insects, air pollution, extreme temperatures, and the like (Tucović et al., 1981). The basic guidelines of reconstruction are the detection and definition of the desirable traits, selection of the initial material, identification of qualitative and quantitative parameters, study of the inheritance method of the selected traits, mass production of the improved plant material, etc.

Material and Method

Acer pseudoplatanus L. is one of the most important species of valuable broadleaves in Serbia. The cultivar «Atropurpureum» is distinguished by the purple undersides of the leaves, which gives purple colour to the crown, especially in the autumn. Thanks to its great ornamentalness, it is grown widely in the parks, green spaces and in tree rows.

The previous studies of the specific phenotype characteristics of this species and its cultivars in Serbia are few. The occurrence of multi-winged fruits on the cultivated sycamore maple trees and the hypotheses of its cause were reported by Ivetić and Tucović, 2003. The heritability of purple leaf underside by the progeny of the cultivar «Atropurpureum» has only been identified in the references (Stilinović, 1987), but it was not the subject of in-depth research.

In the aim of identifying the character of inheritance of purple leaf underside by the progeny of the species *Acer pseudoplatanus* L cultivar «*Atropurpureum*», mother trees were selected and their seeds were collected in the autumn 2005. The trees were selected on the green spaces of Belgrade. In the autumn 2005 the seeds were sown in plastic containers. The colour of leaf underside was analysed on one-year old plants of 9 half-sib lines which developed in environmental conditions. Based on the collected data, the percentage of plants with purple leaf underside, plants with green leaf underside and plants with variegated leaf underside was determined for the progeny of each half-sib line.

Results and Discussion

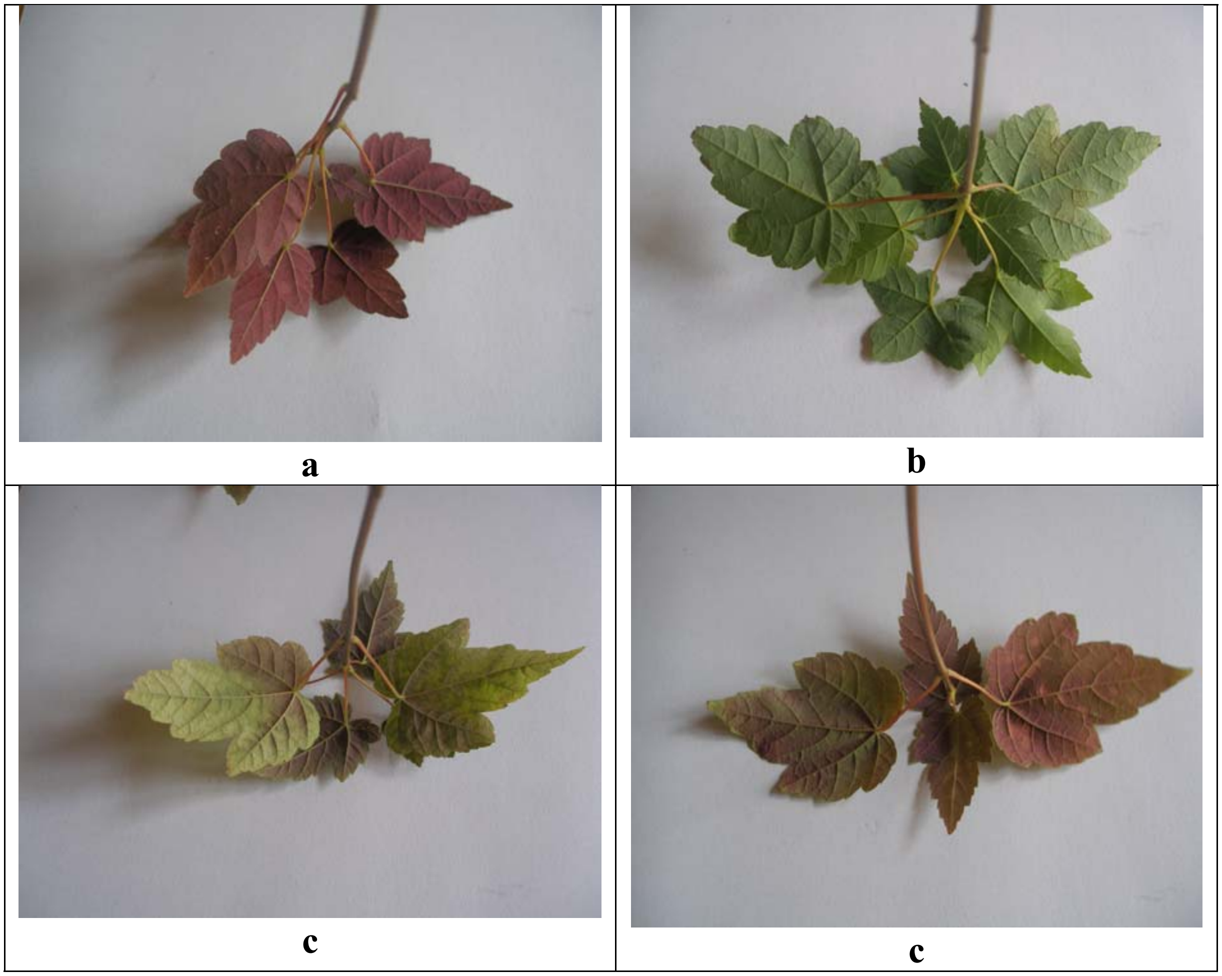
The results of the analysis of the leaf abaxial colour of one-year old plants in 9 half-sib lines, justify the initial hypothesis that the degree of heritability of purple leaf underside is highly variable and determined by genetic constitution of mother trees. The study results show different percentages of the foliage with purple, green and variegated undersides in the obtained progeny, Table 1.

Table 1: Percentage of leaves with purple and green undersides in the total number of leaves of *Acer pseudoplatanus* L. cv. «*Atropurpureum*» half-sib lines and the germination percentage of the seeds of mother genotypes

Half-sib line	Leaves with green underside (%)	Leaves with purple underside (%)	Leaves with variegated underside (%)	Germination (%)
1	10.15	85.87	3.98	82
2	49.79	46.81	3.40	62
3	12.86	81.33	5.81	28
4	50.21	44.44	5.35	60
5	0.00	94.14	5.86	40
6	81.72	17.20	1.08	81
7	74.55	24.37	1.08	91
8	6.98	90.37	2.65	32
9	46.30	52.14	1.56	35

From the aspect of further breeding and commercial production of seedlings with desirable characters, the subject of this and further studies are mother trees whose progeny is distinguished by a high percentage of the leaves with purple undersides, Figure 1a. Such trees are 1, 3, 5, 8 and 9, in which the percentage of such leaves is between 80 and 90% (except tree 9, in whose progeny the percentage is somewhat above 50). In addition to a high percentage of the desirable characters in the obtained progeny, the seed germination percentage of the selected mother trees is also significant, which is a necessary precondition for mass production of seeds and seedlings of this cultivar. According to the references (Stilinović, 1985), germination percentage of fresh seeds is between 70 and 80%. In our research, mother tree 1 is clearly distinguished by a high percentage of the desirable characters in the progeny and by a high seed germination percentage (82%). Other trees with a high germination percentage (trees 6 and 7) produce predominantly leaves with green leaf underside, Figure 1b. In further research, germination percentage should be tested in laboratory conditions, by applying *ISTA* standard, in the aim of the greater preciseness.

Figure 1: Progeny of the selected mother trees of *Acer pseudoplatanus* L. cv «*Atropurpureum*»: a – plants with purple leaf underside; b – plants with green leaf underside; plants with partial percentage of the leaves with purple and green leaf underside



In our research, the leaves with variegated undersides were identified in all half-sib lines, Figure 1c. The percentage of such leaves ranges between 1.08 and 5.86 % which is relatively low, but not negligible, as this is a very specific ornamental characteristic, rare in nature. Consequently, the development of this character, as well as the degree of its heritability, should be monitored at the phenotype and molecular levels, especially in mother trees whose progeny is characterised by such leaves above 5%.

In addition to variegated undersides, there are also plants with simultaneous (on the same individual) occurrence of the leaves with both purple and green undersides in different ratios. Taking into account the very small number of such individuals, they should be monitored during further research.

Conclusions

Mother trees whose progeny is characterised by the percentage of heritability of the study character above 50% and whose seeds are distinguished by high germination percentage should be the base of future breeding programme in the aim of producing the nursery stock with desirable characters. The model of further breeding should include the establishment of the clonal seed orchard of selected mother trees, in which the production of planting material should be intensified.

The existence of plants with the simultaneous occurrence of the leaves with purple and green leaf undersides, as well as the plants with variegated leaves, is extremely interesting because spontaneous occurrence of such phenotypes is very rare in nature. The individuals with variegated leaves are most probably the phenotype expressions of heterozygous genotypes on the gene-locus responsible for the expression of the above character. The explanation of the phenomenon of partial percentage of the leaves with green and purple undersides on the same tree can be the mutation in the DNA nucleus and organelle. As it is a known fact that purple coloured leaves are formed when chlorophyll is masked by anthocyanin, which is synthesised in the greater than typical quantity and that chloroplasts contain the genetic material which determines the cytoplasm heritability, further research should be performed at the molecular level, in order to define the possible laws of the genetic control of the anthocyanin synthesis and phenotype expression. The research of molecular markers inherited by mother trees (chloroplastic DNA) is a reliable base of the identification of the heritability mechanism of the above phenotype. In this way, the direction of breeding of this valuable broadleaf species should also be determined. Also, it is necessary to determine the stability of these specific characters during the plant ontogenetic development, i.e. the possible phenomenon of alternating or delayed dominance should be recorded.

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Pollination Influence on Ginkgo Seed and Seedling Traits

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Abstract

Ginkgo has very wide cultural range in Serbia because of its decorative traits and high adaptability on urban environment. Including breeding program techniques and plant production components, researches with the aim of genetically improved seed and seedling material development have been organized. Estimation of mother trees genetic potential and description of pollination system within an isolated mikropopulation have been based on yield quantity and quality analyzes and seedlings morph metric traits measuring. Defined variability could be explained with different genetic potential of mother trees, pollination system characteristics (available pollen amount) and environmental conditions in masting year. These steps of research work in ginkgo breeding program are very mportant base for seed stems selection.

Keywords: Ginkgo biloba L., breeding program, genetically improved plant material

Introduction

Tree breeding involves the process of reconstruction of natural or cultural populations of individual species to the benefit of the individuals for which man is directly interested. The basic unit of reconstruction is an individual, i.e. its genotype, and the study of individual variability is the base of all breeding processes.

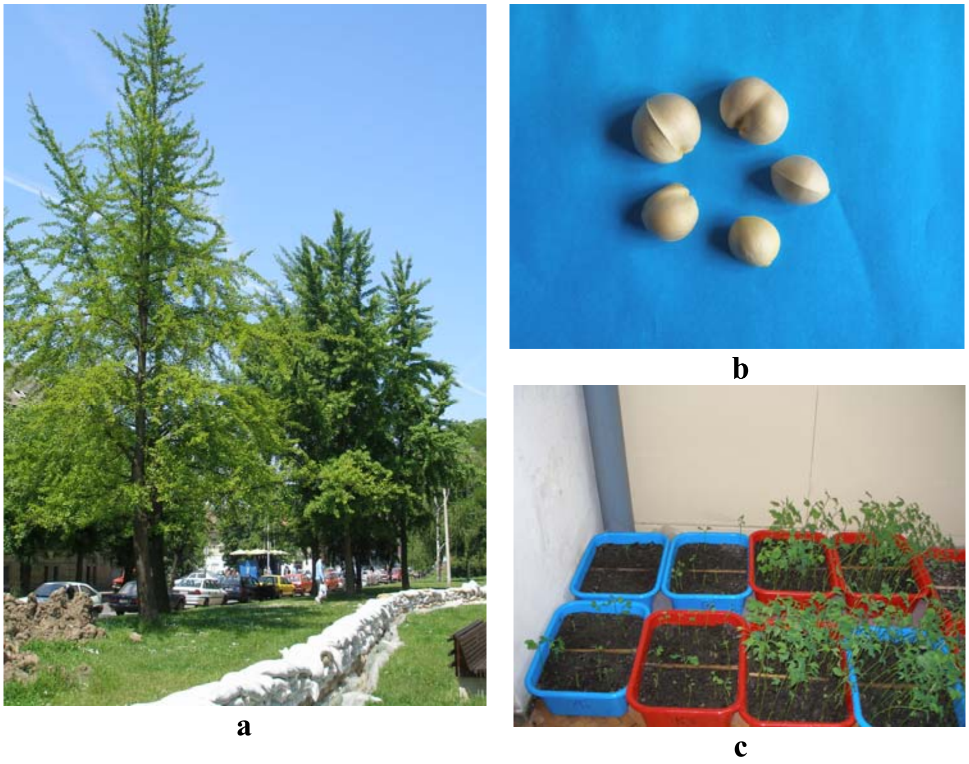
By connecting the issues of tree breeding with the production-technological component of plant production, and by the detection of the superior genotypes, this study aims at the improvement of planting material. The genetic potential of an isolated group of ginkgo mother trees was assessed by the analysis of their characteristics and the pollination method, by the analysis of the yield quantity and quality and the analysis of morphometric traits of the obtained lines in the juvenile phase of development.

Material and Method

Ginkgo (*Ginkgo biloba* L.) is the oldest and the most resistant tree species on the planet. It originates from China and it is about 250 million years old, from the geological era Mesozoic, Triassic period (Vilotić, D. 2004). On the territory of Serbia, ginkgo is an allochthonous species, introduced by man. It occurs on green spaces mainly as individual trees or smaller tree groups, very rarely in tree rows. In the wider area of Belgrade city, after Vilotić, D. 2004, there are 94 ginkgo trees. There are no written records about the origin of ginkgo seeds and seedlings on the territory of Serbia, but it is assumed that the seeds and seedlings were not brought from Asia, and that they are of European origin (from the parks of England, Check Republic, Slovakia, Germany, etc.). Taking into account its multiple benefit value, the interest for this species in Serbia has been growing in the past years.

Our research was performed in the isolated group of ginkgo trees situated in the green space of Zemun Quay. The group consists of five trees in two sets. The first set consists of three trees (marked 1, 2 and 3), from which the trees 1 and 2 are female and the tree 3 is male. The second set consists of two female trees, marked 4 and 5. The trees in these two sets are growing at the distance of 6 to 7 metres and differ by their phenotype characters and the characteristics of leaves, fruits and by the phenophases of leafing, flowering and fructification. Figure 1a

Figure 1: **(a)** An isolated group of ginkgo trees on Zemun Quay; **(b)** Abnormal seed; **(c)** Variability of plants in the juvenile phase of development



In the autumn 2005, the seeds were collected from mother trees 1, 4 and 5. The analysis of the dimensions of clean seeds was performed on the sample of 100 seeds and it includes the following morphometric parameters: seed length (mm), seed width (mm) and seed mass (g). Plant analysis in the juvenile phase of development includes the following parameters: shoot length (cm), root length (cm), number of leaves, leaf mass (g), shoot mass (g) and root mass (g). The collected data were processed by computer programme «Statistika 6.0». The statistical justification of the differences between the mean values of the analysed morphometric characters of seeds and seedlings of mother trees was tested by Student t-test, probability 95% (Isajev and Šijačić-Nikolić 2003).

Results and Discussion

The results of the analysis of morphometric characters of seeds and plants in the juvenile phase of development are presented in Tables 1 and 2.

The seed germination percentage is a character which shows a high variability of the analysed mother trees. The tree 1 has germination percentage 66.04%, germination of the tree 4 has not been recorded at all, and the tree 5 has germination percentage of only 13.20 %.

Table 1: Results of the analysis of seed morphometric characters

Seed length (mm)					
	X	S	t - value		
			1	4	5
1	20,76	0,70	-		
4	16,20	0,62	-47,87*	-	
5	16.79	0.81	-38.59*	5.18*	-
Seed width (mm)					
	X	S	t - value		
			1	4	5
1	16.31	1.93	-		
4	12.09	0.72	-20.15*	-	
5	13.10	0.92	-3.21*	8.13*	-
Seed mass (g)					
	X	S	t - value		
			1	4	5
1	2.28	0.50	-		
4	0.60	0.11	-28.83*	-	
5	0.99	0.19	-20.52*	17.87*	-

Table 2: Results of the analysis of morphometric characters of the plants in the juvenile phase of development

Shoot length (cm)					
	X	S	t - value		
			1	4	5
1	22.35	16.50	-		
4	0	0	60.52*	-	
5	10.67	31.11	14.29*	-12.13*	-
Root length (cm)					
	X	S	t – value		
			1	4	5
1	10.24	9.76	-		
4	0	0	46.91*	-	
5	11.94	35.67	-2.05	-14.96*	-
Number of leaves					

	X	S	t - value		
			1	4	5
1	5.35	0.57	-		
4	0	0	-40.74*	-	
5	4.00	0.64	8.10*	-27.56*	-
Leaf mass (g)					
	X	S	t - value		
			1	4	5
1	0.46	0.16	-		
4	0	0	12.91*	-	
5	0.66	0.22	-3.86*	-13.12*	
Shoot mass (g)					
	X	S	t - value		
			1	4	5
1	1.28	0.19	-		
4	0	0	29.82*	-	
5	1.17	0.34	1.29	-12.18*	
Root mass (g)					
	X	S	t - value		
			1	4	5
1	0.55	0.09	-		
4	0	0	26.40*	-	
5	0.64	0.19	-1.88	-14.57*	-

Based on the results of morphometric analysis of seed characters, it can be concluded that the differences between the mean values are statistically significant for all three analysed characters (Šijačić-Nikolić, M. *et al.*, 2006 a, b). The results show that mother tree 1 has considerably larger seed dimensions than it is the case with the trees 4 and 5. The same refers to seed mass. A number of abnormal seeds was found on the tree 1, Figure 1b.
The results of the analysis of morphometric characters of the plants in the juvenile phase of development show that the differences between the mean values of plant shoot and root masses are not statistically significant. Significant differences between the mean values refer to shoot length, number of leaves and leaf mass. The tree 1 has a twice longer shoot length than the tree 5.

The study results can be explained by the presence and vicinity of a male tree to the mother tree 1, as well as by their being in the same phenogroup. Smaller seed sizes, lower seed mass, poorer germination percentage, or the absence of germination in the trees 4 and 5, may be the consequences of the distance and the lower number of male individuals compared to female trees, which results in the poorer pollination, or in the complete absence of pollination. Also, the explanation can be found in the fact that mother trees 4 and 5 and the pollinator tree belong to different phenogroups, which can result in the incompatibility of parent genotypes.

Conclusion

The results of the research of the ginkgo tree pollination system within small isolated populations, such as occur in the region of Serbia, show that the morphometric characters of seeds and plants in the juvenile phase of development are conditioned by the genetic constitution of mother trees, by the presence i.e. absence of the sufficient amount of pollen, compatibility i.e. incompatibility of parent genotypes and by environmental conditions in the year of seed formation. Also, these micro populations hide in themselves the potential of inbreeding (Stilinović, Tucović, 1975). The aim of our research was to record the mother trees which form large seeds from year to year. The recorded vigorous germination percentage after sowing and the fast growth of plants in the first years of life could have a practical breeding significance. Also, the study results suggest that, in the establishment of small isolated groups of ginkgo trees, attention should be paid to the ratio of male and female trees in the group, their distribution and distance, as well as to the compatibility of parent genotypes. This should result in the successful fructification within the isolated populations, especially if it is born in mind that, for the time being, these are the only potential sources of ginkgo seeds in Serbia.

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Breeding and Conservation of Eucalyptus urophylla an Important Hybrid Parental Species in South Africa

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Abstract

Eucalyptus urophylla S.T. Blake is one of two *Eucalyptus* species that does not occur in Australia. It is found on seven islands in the eastern Lesser Sunda Archipelago of Indonesia. Morphologically, the species is extremely variable at both the provenance and individual tree level with great differences observed in bark, fruit, and leaf morphology. *Eucalyptus grandis* is an important tree species for pulp production in South Africa due to its rapid growth and acceptable wood properties. However, the species suffers from poor survival in the Zululand coastal region because of fungal diseases; in particular *Crysoporthe austroafricana* (formerly known as *Cryophonectria cubensis*) and *Coniothyrium* sp. cankers. Due to its tropical origin, *E. urophylla* has been found to be more disease tolerant than *E. grandis*. Hybrids between the two species have been produced to combine the good survival, disease tolerance and rapid early growth of the parent species. Some of these hybrid clones have produced volume gains of up to 40% greater than commercial *E. grandis* derived from seedlings. Although *E. urophylla* may also have potential as a pure species, it is currently the main partner in the successful *E. grandis x E. urophylla* hybrid and is therefore an extremely important parental species in Sappi's Hardwood Hybrid Breeding Programme. From 1996 to 2003, CAMCORE at North Carolina State University and one of its members, PT Sumalindo Lestari Jaya, collaborated on a new series of collections of *E. urophylla* in Indonesia. A total of 62 native provenances and 1104 mother trees were sampled from all of the seven islands where the species has been identified. Sappi has established a subset of 458 of these half- sib families, from 27 provenances, in five structured sub-populations. *E. urophylla* is one of five *Eucalyptus* species that is actively being bred by Sappi and must compete for financial resources with the other species. This presentation will demonstrate the importance of *E. urophylla* as a hybrid parent, outline some of the challenges for its development such as pollen contamination from other eucalypt species, that makes following an open-pollinated low cost breeding strategy difficult. Opportunities will also be explored for the conservation of this valuable and endangered resource.

Keywords: *E. grandis,* disease tolerance, provenance, volume gains, clones, survival, pollen contamination

New Breeding and Deployment Strategy Using Conifer Somatic Embryogenesis and Pedigree Reconstruction

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Abstract

In the past 30 years, tree improvement programs around the world have contributed greatly to forest productivity through plantation forestry. Conventional tree improvement programs are usually long-term efforts involving multi-generation recurrent selection. These programs are generally expensive and are often conducted cooperatively among a number of participating agencies. In a small-scale reforestation program or a new program, however, the cost of carrying out long-term breeding and testing as well as managing seed orchards can be a major limiting factor. In designing these tree improvement programs, it is important to consider the practical goals such as: (1) obtaining greater genetic gain; (2) flexibility to adapt to changing conditions; and (3) ability to manage genetic gain and diversity. Owing to recent developments and refinements in somatic embryogenesis (SE) and molecular marker technology, a new breeding, testing and deployment strategy is proposed. This new strategy, proposed for its simplicity and efficiency, is applicable to both new and advanced generations as well as small-scale programs.

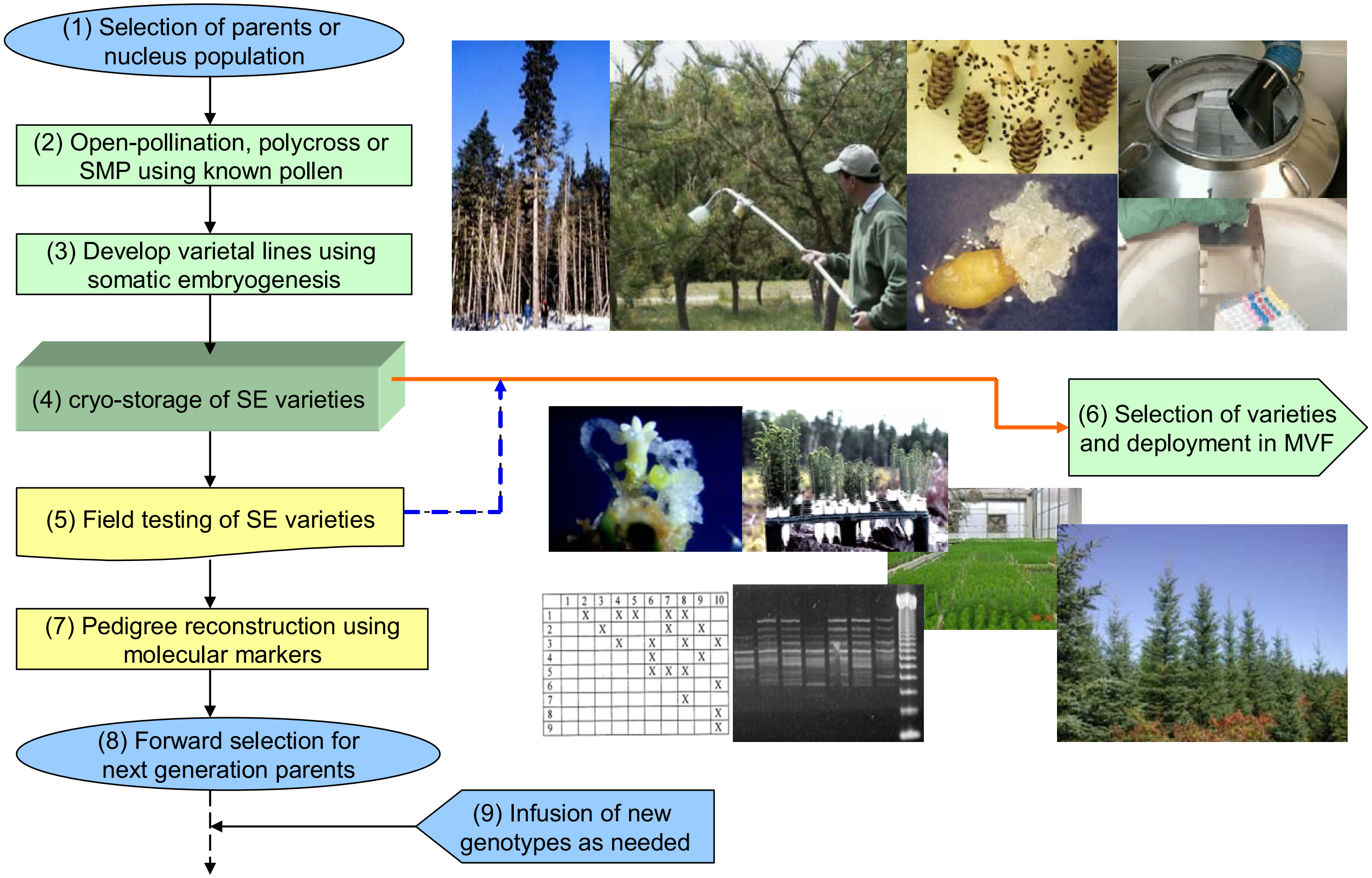
There are two main elements. The first is implementation of Multi-Varietal Forestry (MVF), i.e., the use of tested tree varieties in plantation forestry, which can deliver the greatest genetic gain without seed orchards. The second is "Breeding Without Breeding (BWB)" that involves the construction of pedigree without laborious controlled-pollinations. A method has been developed for pedigrees construction from open-pollinated, polycross and supplement mass pollination (SMP) progenies using molecular markers. The strategy is outlined in Figure 1.

The focus of this presentation is to illustrate the utility of MVF on a commercial scale using spruce species in eastern Canada as a model. The MVF strategy will be discussed, including technical requirements for SE, maximizing genetic gain, and the management of plantation genetic diversity. Relevant data from clonally replicated genetic tests will also be presented.

Key words: Cryopreservation, microsatellite, pedigree reconstruction, nucleus population, forward selection,

Figure 1

Breeding and deployment strategy using somatic embryogenesis and molecular markers



(1) Selection of parents or nucleus population

(2) Open-pollination, polycross or SMP using known pollen

(3) Develop varietal lines using somatic embryogenesis

(4) cryo-storage of SE varieties

(5) Field testing of SE varieties

(6) Selection of varieties and deployment in MVF

(7) Pedigree reconstruction using molecular markers

(8) Forward selection for next generation parents

(9) Infusion of new genotypes as needed

	1	2	3	4	5	6	7	8	9	10
1		X		X	X		X	X		
2			X				X		X	
3				X		X		X		X
4						X			X	
5						X	X	X		
6										X
7								X		
8										X
9										X

Low Cost Strategies for Domesticating and Breeding Trees

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Abstract

The development of a tree breeding strategy of any kind requires consideration of a number of factors, which apply equally to simple and sophisticated, large and small scale programmes. These include the products for which the species is grown; gene resources and selection; species biology (e.g. reproductive and vegetative propagation characteristics); breeding knowledge (breeding objectives, selection criteria, genetic parameters); pedigree identification level; scale (numbers of plants to be trialled); breeding efficiency (gain per year per dollar) and technology level (high or low tech). Maintenance of pedigree, either of both parents, female parent only, or not at all, is a key element determining cost. Thereafter the cost is largely determined by number of families and individuals in field tests. Low cost strategies are often required to be simple and low tech, depending on economic and technological development, and funding. The species' biology, whether exotic or native, is crucial to the strategy. Some small-scale and low-cost breeding and seed production strategies were therefore devised and their gains simulated deterministically. A breeding population of 25 open-pollinated families, planted on three test sites, managed by balanced within-family forwards selection, and accompanied by a 10-clone seed orchard, can give acceptable levels of genetic gain, depending on trait heritability, that will be sustainable over two or three generations. Cloning of juvenile seedlings of this population, if the species is easily propagated vegetatively, can lead to high gains from tested clones in clonal forestry deployment. Grafting selections into a clonal archive cum clonal orchard, with collection of open-pollinated seed there for breeding population cycling, gives improved gain over collection of seed for this purpose from family tests. The simplest and cheapest means of cycling the breeding population and producing improved seed that gives gains approaching those of pedigreed populations, is by planting stands of bulked open-pollinated families of phenotypically-selected parents, with commercial seed collection from felled selected trees in plantation stands.

Keywords: pedigree, gain, deterministic simulation, seed production, cloning

Towards possibilities of some exotic *Abies* species use in the Czech Republic forestry practice on the base of evaluation of their progenies growth on the locality Jiloviste – Cukrak in Central Bohemia at the age of 30 years

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Abstract

In this report, there are presented results of evaluation of provenance research plot No. 58 – Lesy Jiloviste, Cukrak, which was established in 1975. Extensive number of various *Abies* species is represented in this research plot. The aim of presented report is to resume results of evaluation at the age of 30 years. Evaluated progenies of *Abies grandis* are characterized by growth over average value and by average mortality. Some positive, as well as some negative characteristics, have been found out in case of *A. balsamea* and *A. concolor*. Progenies of *A. borisii-regis* and *A. nordmanniana* have been characterized as very promising. As for *A. pinsapo*, *A. cilicica*, *A. magnifica*, *A. bornmulleriana* and *A. lasiocarpa*, these species have been identified as not applicable for the Czech Republic forest management.

Keywords: *Abies cilicica*, *A. cephalonica*, *A. grandis*, *A. balsamea*, *A. pinsapo*, *A. concolor*, *A. nordmanniana*, *A. lasiocarpa*, *A. fraseri*, *A. magnifica*, *A. bornmulleriana*, introduction, exotic tree species, provenance research.

Rapid improvement approach for Teak (*Tectona grandis*), Mahogany (*Swietenia macrophylla*) and Deglupta (*Eucalyptus Deglupta*) in the Solomon Islands

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Natural forests in the Solomon Islands have been heavily logged over the last 15 years and current forest inventories indicate that resources will be depleted within about five years. In order to provide source of income for families for the future, an AusAid (Australian Government Aid) Forest Management Project has been encouraging villagers to establish their own plantation/woodlots. Initial emphasis has been on Teak and Mahogany with approximately 3,500 ha established over the last 3 years. Additionally, there has been recent interest in establishing stands of Deglupta. To ensure that only high quality material was planted in the future, a breeding strategy for each of the three species was initiated in 2004.

The limited time frame in which to produce seed, uncertain genetic basis of most trial plots from which trees could be selected for breeding and a low budget, required innovative measures. There was no time to import new germplasm and travel was difficult and costly, so the best trees of each species were selected from all stands and plots of each species on Kolombangara and New Georgia islands only. Unfortunately, flowering can be sporadic across years for some trees and access difficult for others so not all of the best trees were included.

The strategy used for each species was to include all of the best trees chosen which had seed in the year of collection, in open pollinated progeny tests which would then be converted into seedling seed orchards.

By November 2006 there will be 8 ha of progeny trials of each species. Each species will have row-column designed progeny trials across three sites with 80 families in the teak, 48 families in the Mahogany and 56 families in the Deglupta. At age 3.5 years the Teak and Deglupta trials will be culled to the best 60% of ranked families and plots thinned to the best tree to create seedling seed orchards. At this age trees will be 16-18 m tall with diameters around 20 cm. These Teak and Deglupta seedling seed orchards are expected to begin producing improved seed by 2010. Mahogany does not flower until age 12 years, and therefore, improved Mahogany seed will not be available until 2018.

Key Words: Seed orchard, Breeding, Teak (Tectona grandis), Mahogany (Swietenia macrophylla) and Deglupta (Eucalyptus Deglupta), Solomon Islands

Breeding strategy with low input for fast improvement of *Tectona grandis* (teak), *Swietenia macrophylla* (mahogany) and *Eucalyptus deglupta* (deglupta) in the Solomon Islands

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Abstract

Natural forests in the Solomon Islands have been heavily logged over the last 15 years and current forest inventories indicate that resources will be depleted within about five years. In order to provide future sources of income for families, an AusAid (Australian Government Aid) Forest Management Project has been encouraging villagers to establish their own plantations/woodlots. Initial emphasis has been on *T. grandis* and *S. macrophylla* with approximately 3,500 ha established over the last 3 years. Additionally, there has been recent interest in establishing stands of *E. deglupta*. To ensure that only high quality material is planted in the future, a breeding strategy for each of the three species was initiated in 2004.

The strategy was to develop local genetic improvement programs for these species. The immediate demand for seed, uncertain genetic basis of most trial plots from which trees could be selected for breeding and a low budget, required innovative measures. Timely importation of new germplasm was unfeasible, and travel was difficult and costly, so an alternative strategy was required.

The breeding population for each species was formed in a series of progeny trials based on best available phenotypic selections (candidate plus trees) which had seed in the year of collection. The progeny tests will later be converted into seedling seed orchards. The candidate plus trees of each species all originate from stands and plots on Kolombangara and New Georgia Islands, where approximately 90% of all trials and plots were established.

By November 2006 there will be 8 ha of progeny trials of each species. Each species will have row-column designed progeny trials across three major planting sites with 79 families of *T. grandis*, 48 families of *S. macrophylla* and 80 families of *E. deglupta*. At age 3.5 years the *T. grandis* and *E. deglupta* trials will be culled to the best 60% of ranked families and plots thinned to the best tree to create seedling seed orchards. At this age trees are anticipated to be approximately 16-18 m tall with diameters at breast height around 20 cm. The *T. grandis* and *E. deglupta* seedling seed orchards are expected to begin producing improved seed by 2010. *S. macrophylla* does not flower until age 12 years, and therefore, improved *S. macrophylla* seed will not be available until 2018.

Key Words: Seed orchard, breeding,

Introduction

Natural forests in the Solomon Islands have been heavily logged over the last 15 years and current inventories indicate that forest resources will be depleted within about five years. This has caused concern as approximately 40% of the Government's revenue has been derived from royalties and other taxes associated with forest logging. The specific impact on incomes of families that are selling the trees from their land is also of particular concern. As a consequence, the AusAid funded project "Forest Management in the Solomon Islands (FMP)" initiated an extension programme to encourage growers at the village level to establish their own plantations/ woodlots as a source of future income.

The choice of *T. grandis, S. macrophylla* and *E. deglupta* was based primarily on fast growth rates across a range of soil types, increased international demand for plantation-grown cabinet timbers and price per unit volume at sale.

Because *T. grandis* seed can be stored and planted when convenient, growers are trained to germinate, grow and stump *T. grandis* seedlings for planting in small individual family woodlots. *S. macrophylla* seed loses viability fairly quickly so seedlings are raised by the Forestry Department (FD) and sold to growers at subsidised rates. The extension programme also provides advice on identifying suitable planting sites, spacing and management for each species.

Current seed sources for *T. grandis* consist of an older stand that has been heavily thinned for seed production and a clonal archive of a single ramet from each of 45 clones selected from across all trials in the Solomons in 1990. *S. macrophylla* seed is collected from individual plus trees and bulked, while *E. deglupta* seed has been difficult to procure from Papua New Guinea on a regular basis and is therefore only collected from local plus trees and bulked as required.

Initial emphasis has been on growing *T. grandis* and *S. macrophylla* with approximately 3,500 ha being established since 2001. *E. deglupta* was added to the overall programme in 2004. Additional impetus to produce seed of a higher quality than is currently available is provided by the Forestry Division, Ministry of Forestry and Environment, Solomon Islands (FD) and Forest Management Project (FMP) outgrower extension programme and two private commercial plantation owners (Kolombangara Forest Products limited (KFPL) and Eagon Pacific Plantations Limited (EPPL)).

To ensure that better quality material will be planted in the future, a breeding strategy common to each of the three species was developed in 2004. This paper discusses the low input strategy used to achieve a genetic improvement and seed production in a short time frame for the three species.

Genetic resources

Uncertainty as to the exact origin of most trial plantings for each of the species made the task of selecting candidate plus trees to form a breeding population with sufficient genetic diversity difficult. Some of the introductions have been identified in the field (Table 1) with plot sizes ranging from 7 -50 trees

Table 1. Introductions of each species to Solomon Islands and plantings still remaining and able to be identified by introduction in 1989

Species	First Planted	Total No. seedlot Introductions 1957-1989	Introductions by Country	No. identified plantings by introductions in 1989	Reference
T. grandis	1957	24	India (9)	5	Sandiford 1990a
			Thailand (2)		
			Burma (1)		
			PNG (4)	3	
			Samoa (2)	1	
			Trinidad (2)	1	
			Indonesia (2)		
			Nigeria (2)		
S. macrophylla	1957	40	Fiji (35)	8	Sandiford 1990c
			Philippines(2)	2	
			Trinidad (1)	1	
			Honduras (1)	1	
			SriLanka (1)	1	
E. deglupta	1957	49	PNG (33)	7	Sandiford 1990b
			Indonesia (5)	1	
			Phillipines (2)		
			Costa Rica (3)		
			Cuba (1)	1	
			Samoa (1)		
			Australia (4)	1	

Until the late 1970s, many trial plantings were unstructured plots, the genetic origins of which can only be guessed at. Introductions from countries where a species is exotic have been assumed to be from mature-age trial plantings or possibly seed production areas (SPA) or, as in the case of Australia, transhipped from another source. Initial introductions of *S. macrophylla* from Fiji are more than likely from SPAs, and more recently from clonal seed orchards (P. Zekele, *pers comm*. 2005)[2].

Solomon Islands Forestry Division records indicate that some seed collections were made from identified plots for all three species, however, most seed was not sown. For *T. grandis*, most trial plantings in the last 30 years were derived from 5 of the identified sites in the Solomons though the number of parents involved at each collection is not recorded. The pattern is similar for *S. macrophylla* where most plantings in the last 30 years were from seed collected in 4 small plots at Mt Austin on Guadalcanal Is and bulked. Continued introductions from Fiji helped meet demand. Most of the *E. deglupta* seed came from SPAs in PNG and since 1976 the clonal seed orchard (19 single clones) at Bulolo. Of the 15 natural stand collections introduced, only several remain identified and are not used as seed sources.

Current seed sources

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[2] P. Zekele, Deputy Research Director, Forestry Division, Ministry of Forests and Environment, Solomon Islands.

T. grandis**:** In the early 1990s, a *T. grandis* clonal archive was established by Basil Gua, Research officer, FD The clonal archive contains a single graft from each of the best 50 plus trees in the Solomons selected in 1990. In 2000, an abandoned thinning trial was identified as a suitable seed production area (SPA) and was lightly thinned to improve the quality of the seed collected. These two areas have been the principal sources of seed for supply to extension growers and will continue to be until the new seed orchards produce in 2011.

The quality of the seedlings derived from the clonal archive is vastly superior to the seedlings from the SPA. Measurements of trees in private plantings indicate that the clonal archive seedlings will yield conservatively 20% more volume than SPA progeny. To improve the SPA quality, a further 50% of the existing trees were removed in November 2005 with emphasis on removing the poorer formed trees. However as the genetic relatedness between trees in the SPA is unknown, and may even be the progeny of a single tree, even this measure may not substantially improve matters.

S. macrophylla**:** Seedlings for the extension growers programme managed by FD/FMP are currently raised from seed collected from around 10-15 selected candidate plus trees. Plantation quality, on average, should improve next year with a wider number of plus trees to draw from and an awareness of the importance of bulking seed from individuals before sowing to increase genetic diversity in plantings. This source will not change until the seed orchards come into production in 2018.

E. deglupta: Availability of seed from Papua New Guinea is not reliable so EPPL (whose plantation estate is predominantly *E. deglupta*) collects seed from plus trees in young plantations while KFPL also collects seed from selected plus trees and buys seed from FD. FD collects from more mature plus trees in trial plots. The new seed orchards should come into production in 2011.

Overall Strategy

In the absence of qualified information, all plantings from which selected trees were chosen were assumed to be from bulk provenance collections (natural stand or seed production area) with an unknown number of parents involved in each bulk. The safest option was to assume that the genetic base for each species was reasonably narrow and that all the seedling seed orchards are just the first step in improvement.
Some introduced provenances of *T. grandis* and *S. macrophylla* were so poor in growth and form that they were excluded in the search for plus trees.

The basic strategy chosen was to include all candidate plus trees that had seed at the time of collection and establish them as open pollinated seedlings in replicated progeny tests across the three soil types most likely to be used for commercial plantings. The progeny tests will be thinned to produce seedling seed orchards based on family performances. At age 3.5 years all families for *T. grandis* and *E. deglupta* will be ranked across sites and the top 60% retained with 5-tree row plots being thinned to the best tree in addition. As there is no demand for large volumes of seed, collection will be from only the best 20 families with the others contributing pollen. *S. macrophylla* will be evaluated in the same way but at age 10 years, two years before flowering commences. The risk that you may end up with non-flowering individuals retained is accepted. However, since almost all selections in each species have been cloned into archives, the FD and plantation growers have the future option of creating a longer term improvement strategy with potential further gains by including all progeny in longer term progeny trials at routine spacings, cloning of family selections into clonal seed orchards and importing new germplasm to create a more diverse breeding population.

The low numbers of selections available for inclusion in each of the progeny tests led to the adoption of the strategy of making the breeding population the production population. This is a short term strategy to ensure seed delivery sooner and may restrict further improvement unless selections from elsewhere are infused when the generations are turned over. Clonal seed orchards might produce seed a year earlier but were discounted due to the lack of technical skills needed for grafting.

Without estimates of heritability for these traits in this population, predictions of gain are not possible. Measurements in the seedling seed orchards before selection and thinning will provide data for such estimates. The seedling seed orchard strategy itself has been shown to be effective for other species (Tibbits & Hodge 1995, Barbour & Butcher 1995).

The cloning option or mass vegetative propagation is particularly attractive for *T. grandis* as non-additive genetic variance can represent 35% to 50% of total genetic variance for growth traits, and 63% of total genetic variance for incidence of flowering (Callister & Collins 2006). Such estimates are not yet available for Solomon Islands populations which might represent a narrower and different genetic base.

Factors such as the uncertainty of provenance identification and the numbers of parents in previous collections with possible relatedness does include a risk element of inbreeding which will only manifest in future plantings. This will be minimised during the culling within the progeny tests when further information about relatedness of selected individuals should be known from archived research files.

Progeny trials

For each of the species, the progeny tests consist of 5-tree row plots of each family in a row column design with four replicates at each of three major sites and an alpha-design (a form of incomplete block design) with four replicates at the three minor sites. Initial spacing is 5m between rows x 3m within rows (667 stems/ha). This should result in an average of 15 m x 15 m (333 stems/ha) after culling. Particular attention was paid to laying out the trials so that the total area was as square as possible to facilitate maximum inclusion of all individuals during pollination.

The *S. macrophylla* progeny trials were established in two successive years on each site due to the lack of seed on many selected mother trees in the first year. The first year's planting was at 10 m x 3 m spacing. The second year's planting (with ties to the first year via seedlots from common mothers) was planted in the inter-row space, effectively giving 5 m between rows with alternating rows being planted in different years. The progeny tests will be ranked by family and then culled to create a seedling seed orchard.

Details by Species

T. grandis

The objective was to phenotypically select up to 100 outstanding trees from all known plantings, including the 45 remaining trees in the clonal archive, and use this as the base population for the new progeny trial/SSO. Seed was collected from 79 families (Table 2).

Progeny tests will be measured for height and diameter and assessed for straightness, precocious flowering, stem fluting and branching then thinned to the top 60% of families to create seedling seed orchards before the first general(>50%) flowering period at age 4. Seed will be collected for distribution only from the top 20 families after the age 5 flowering in 2011. It is anticipated that the new seed orchards will provide quality seedlings capable of producing at least a 15-20% increase in volume production/unit area. This will be validated in future yield trials comparing seed sources.

Table 2. Location and areas planted to *T. grandis* as progeny trials which will be converted to Seedling Seed Orchards

Location	Province	Collaborator	No. Families	Area
Poitette	Western	FD/FMP	79	2.65 ha
Ringi	Western	KFPL	77	2.65 ha
Arara	Western	EPPL	77	2.65 ha
Kirakira	Makira	FD/FMP	40	1.0 ha
Buala	Isobel	FD/FMP	40 (grafted)	1.0 ha
			Total area	9.95 ha

Base fluting and sometimes stem fluting can cause serious degrade to teak logs so this character was strongly selected against during plus tree selection though heritability (h^2) is unknown. Early flowering has been estimated to be as high as 20% among individuals in 4-year-old trees on Kolombangara Is. Callister & Collins (2006) estimated that about 15% of trees were flowering by 3.5 years in their trial, yet the estimate of heritability for flowering was low (h^2=0.1). Both observations are at odds with observations by Kaosa-ard (1998) who found that 20-25 years to first flowering was common. Individuals flowering in the seedling seed orchards before age 4 years will be strongly selected against when culling the progeny test as flowering causes the crown to break with loss of apical dominance, limiting log length in the future. Stem straightness, while important in the selection process, does not appear to be under strong genetic control in Australia (h^2=0.15) (Callister & Collins 2006) however a phenotypic correlation of 0.37 and a genetic correlation of 0.73 between diameter and stem straightness in Indonesian populations (Danarto & Hardiyanto, 2000) indicates emphasis on diameter in selection is warranted.

S. macrophylla

Most of the *S. macrophylla* established in the Solomon Is. came from Fijian introductions over a period of 40 years. No previous improvement work had been carried out on *S. macrophylla* in the Solomon Is. other than selecting a few better trees for seed collection, so all plantings were inspected in 2005 and 57 plus trees identified. Seed was available from 48 trees only as seed set is variable among trees across sites. All seed was sown, including a Fijian clonal seed orchard seedlot which was imported in 2005. Seedlings were raised and established in progeny trials on three major sites and two minor sites (Table 3). *S. macrophylla* does not flower until it is 12-years-old so the trials will be monitored for growth and thinned to the best individuals in 30 families by age 10 so as to be ready for seed production by age 12.

Table 3. Location and areas planted to *S. macrophylla* as progeny trials which will be converted to Seedling Seed Orchards

Location	Province	Collaborator	No. Families	Area
Poitette	Western	FD/FMP	48	2.7 ha
Ringi	Western	KFPL	48	2.7 ha
Arara	Western	EPPL	48	2.7 ha
Kirakira	Makira	FD/FMP	27	1.0 ha
Buala	Isobel	FD/FMP	27	1.0 ha
			Total area	10.1 ha

E. deglupta

Most *E. deglupta* seed sown in the Solomon Is. was derived from PNG sources. A few smaller introductions from the Philippines were made in the late seventies to early eighties. *E. deglupta* peels easily and yields good quality veneer. This will be the main use of the resource in the future.

Of the 75 plus trees selected, most originated from PNG sources. Four Philippine introductions were deliberately included as this provenance is known to be resistant to the leaf fungus *Agrilus opulentus* Ker.(Luton & Roberts 1980). It was recognised that the genetic base could be narrow, but having knowledge of the PNG Forest Research Programme that was conducted during the 1965-1980 period (Davidson 1973a,1973b,1973c), there is reasonable confidence that there will be enough genetic diversity in the existing Solomon Island population to avoid serious levels of inbreeding in the first generation. Gains in volume, stemform and wood quality can be made (Davidson 1972) and the new seed orchards will provide a quality source of seed until more genetically-broader introductions are made and evaluated.

Later this year approximately 9 ha of progeny trials will be planted and at age 3.5 years they will be measured for height and diameter and assessed for straightness, stem fluting, spiral grain, density and branching then thinned to the best individuals of 30-40 families. Seed has been collected from those plus trees producing seed this year and was sown in August 2006 (Table 4). *E. deglupta* flowers from age 2 years but seed will not be collected from the seed orchards for deployment until 2011 and then only from the top 20 families.

Table 4. *E. deglupta* seed sources and numbers of families

Location	Collaborator	No.Families
Kolombangara	FD & KFPL	34
Arara	EPPL	41
Bulolo	PNG FRI	5

The Solomon Is Government and the Papua New Guinea Government have agreed to exchange biological material of selected plus trees. The objective is to benchmark the quality of the Solomon Island landrace.

Conclusion and further work

This program is satisfactory for a 1st generation but, if any of the collaborators want to further improve the genetic quality of their deployment material, they will need to broaden the genetic base of selected species with infusions of new germplasm from natural stands and other breeding programmes from overseas All new introductions will need to be evaluated against the best families currently being used as improved material from another program may not perform as well when established "off site".

More expensive programs may include dedicated progeny tests that can be kept for longer without thinning, but the overall budget of this program meant that this was not practicable at present and probably not necessary until further genetic improvements are planned. Traits like wood properties are much more expensive and difficult to assess, but could usefully be added in later generations.

Clonal deployment and/or Clonal Seed Orchards could yield further gains in production in *T. grandis* and *E. deglupta* but skills development in vegetative propagation and grafting is needed first and selection of clones without genetic information would merely be phenotypic. The seedling seed orchards will provide suitable information and material for clonal selection and deployment when these skills become available.

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Low Input Breeding and Genetic Conservation of Pacific Islands Forest Tree Species

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Abstract

This paper provides an overview of the genetic conservation and tree domestication work undertaken in the Pacific Islands through the AusAID-funded South Pacific Regional Initiative on Forest Genetic Resources (SPRIG). SPRIG was undertaken in two phases, over a period of eight years, between 1996 and 2006 mainly in Fiji, Samoa, Solomon Islands, Tonga and Vanuatu. SPRIG developed a Code of Conduct for sharing germplasm and has been recommended as a model for regional co-operation in conservation and research and development of forest tree species. In mid-2006, the Secretariat of the Pacific Community (SPC) assumed responsibility for coordinating forest genetic resources work in the Pacific Islands region and activities have been extended to 22 island countries and territories.

Strategies and management plans for conserving the genetic resources of 15 priority native tree species have been developed and are in various stages of implementation by national partners and local communities in eight nations (Cook Islands, Fiji, Kiribati, Niue, Samoa, Solomon Islands, Tonga and Vanuatu). Domestication and low-input tree breeding activities, including seed collections and multi-function field plantings for the purposes of assessment, genetic conservation and seed production have been undertaken for more than 20 mainly indigenous Pacific Islands tree species in six nations (Australia, Fiji, Samoa, Solomon Islands, Tonga and Vanuatu). Projected economic benefits from these low input breeding activities, both for smallholder tree growers and national economies, are reported in this paper.

Keywords: ***Endospermum*, *Pandanus*, *Santalum*, *Swietenia*, *Tectona*, *Terminalia*,** domestication

Introduction

Pacific island trees species, and the genetic diversity in them, are increasingly threatened. The main threats are associated with habitat loss and change (due to human activities and invasive plant species), agrodeforestation (loss of trees in agricultural systems), overharvesting and climate change (especially more severe El Nino events and associated droughts and uncontrolled fire).

The South Pacific Regional Initiative on Forest Genetic Resources (SPRIG), was a regional development assistance project in the period 1996-2006, and funded by the Australian Agency for International Development (AusAID). SPRIG's goal was *to help Pacific Island countries to conserve, improve and better promote the wise use of the genetic resources of priority regional trees species to enhance environmental protection and to promote economic and rural development*. The project assisted Pacific Island nations, especially Fiji, Samoa, the Solomon Islands, Tonga and Vanuatu, in conserving and improving priority forest tree species. SPRIG was managed by an Australian consortium (ENSIS, Queensland Department of Primary Industries and URS Sustainable Development) in collaboration with national forestry departments and regional organizations, notably the Secretariat of the Pacific Community (SPC), the Secretariat of the Pacific Regional Environmental Program (SPREP) and the University of the South Pacific (USP).

The main technical components of SPRIG were conservation and tree improvement; these activities being underpinned by a strong institutional-strengthening and capacity-building programme. SPRIG mainly focused on indigenous Pacific Islands tree species, which had previously largely been neglected in forestry R&D and plantation programmes.

Results and Discussion

SPRIG assisted in development of conservation and sustainable management plans for the following Pacific Islands tree species: *Agathis macrophylla, A. silbae, Canarium harveyi, C. indicum, Dacrydium nausoriense, Endospermum medullosum, Garcinia sessilis, Intsia bijuga, Manilkara samoensis, Pandanus tectorius, Santalum austrocaledonicum, S. insulare* var. *mitiaro, S. yasi and Terminalia richii* and *Xanthostemon* (an undescribed species from Solomon Islands).

The conservation plans developed by SPRIG partners (e.g. Community of Mitiaro *et al.* 2004, Corrigan *et al.* 1999., Pouli *et al.* 2002, Havea *et al.*) have been highly participatory, with involvement of concerned stakeholders including especially the local land and resources owners. They are also practical, taking into account the limited additional resources which are likely to be available for their implementation. Individual species conservation plans have promoted an integrated approach to conservation of populations, involving complementary *in situ* and *ex situ* approaches, including conservation through sustainable use in native forests and through domestication and planting in diverse agroforestry systems. These strategies are in various stages of implementation by project partners. A major weakness is that they have, in most cases, been developed in the absence of information on the distribution of intraspecific genetic variation: accordingly they have not been able to focus the conservation measures in areas of higher or unique diversity in the target species.

Indigenous Pacific Islands tree species under investigation and improvement include *Canarium harveyi, C. indicum, Cordia subcordata, Endospermum medullosum, Endospermum robieannum, Flueggea flexuosa, Garcinia sessilis, Gmelina moluccana, Manilkara samoensis, Pleiogynium timorense, Pandanus tectorius, Pometia pinnata, Pterocarpus indicus, Santalum austrocaledonicum, S. yasi, Terminalia catappa, T. richii,* and *Vitex cofassus*. Simple tree improvement plans have also been developed and initiated for selected priority exotic tree species including mahogany (*Swietenia macrophylla*), teak (*Tectona grandis*), red cedar (*Toona ciliata*) and kamerere (*Eucalyptus deglupta*).

The low input breeding approaches used in SPRIG have varied depending on species and resources, but typically entail range-wide (or extensive) germplasm collections, followed by provenance/family trials, mass or family based selection, and development and implementation of effective germplasm multiplication and propagation strategies (both seed-based and clonal). These simple, but robust approaches have resulted in substantial genetic improvement for the major planted tree species in the Pacific Islands. Examples of the results achieved and anticipated economic impacts and benefits, both for individual tree growers and for national economies, are discussed below.

Endospermum medullosum **(whitewood)**

Endospermum medullosum is a useful timber species with outstanding forestry plantation and agroforestry potential in the South Pacific region (Thomson 2006). It occurs naturally in lowland, humid tropical climates on the island of New Guinea, extending through the Solomon Islands to Vanuatu as far south as the island of Erromango. In Vanuatu, whitewood grows rapidly, has high cyclone resistance, and its timber is in high demand, both locally and for export to Japan. Accordingly, the Department of Forests has encouraged planting of the species by small farmers through provision of seedlings and silvicultural information. The Department also nominated whitewood as a top priority for improvement in the SPRIG program.

In 1998, seed was collected from more than 154 whitewood individuals in 16 provenances from throughout the natural distribution in Vanuatu. Provenenance/progeny trials, using row-column designs, were established on 10 ha at the Shark Bay Field Research Station on Santo, Vanuatu. In the Shark Bay field trials the 7 best families (mainly originating from south-east Santo) have grown rapidly during the first four years at a rate of 18 to 23 m^3 ha^{-1} yr^{-1}. The average for all Vanuatu material was 15 m^3 ha^{-1} yr^{-1}. Heritability for volume production was 0.20 (Viji 2004). Therefore the progeny of the most productive open-pollinated families were growing about 5 m^3 ha^{-1} yr^{-1} faster than the average. Based on a stumpage of \$US 35/m^3 (industry estimate for plantation-grown whitewood) this represents an additional annual return to individual plantation growers using improved whitewood of \$US 350-875 for the average larger grower with an area of 2 to 5 ha

The returns to the Vanuatu economy of using improved whitewood germplasm developed under SPRIG are considerable. It is anticipated that over the next decade that the remaining IFP site (5,000 ha) and an equivalent area of private plantings (5,000 ha) will be planted to improved whitewood sources. Once fully planted the increased annual whitewood production from using selected and improved sources will be 50,000 m^3. Given a sawn timber recovery rate of 40% this equates to 20,000 m^3 of sawn whitewood for export with, a conservative estimate, of an additional annual export revenue of \$US 8.5 million to Vanuatu economy (based on \$US 423 per m^3 FOB).

Swietenia macrophylla **(big-leaf mahogany)**

Big-leaf mahogany is one of the most valuable and highly sought after tropical timbers. International markets for mahogany timber are strong and likely to remain so, especially given the decline in supply from native sources in central and South America. Fiji has a major comparative advantage for growing mahogany in that it is free of *Hypsipyla* species (mahogany shoot borer) which causes extensive damage to mahogany plantation throughout the tropical world. Mahogany is the main plantation hardwood grown in Fiji: the total area of plantation mahogany is currently about 42,000 ha.

In 1999 a mahogany provenance/progeny trial was established at Nukurua (SE Viti Levu, Fiji) as part of a joint research effort by the Department of Forestry and SPRIG/AusAID. This provenance/progeny trial included 80 seed sources: 77 from different South American countries (Costa Rica – 28 o.p families, Mexico – 22 o.p families, Honduras – 9 o.p. families, Panama – 18 o.p. families); and 3 bulked seedlots from planted Fiji sources.

Preliminary assessment results after 3.0 years indicated highly significant differences (<0.01) among the progenies in both height and survival, with the tallest families originating from Costa Rica and Honduras. The early growth of these seed sources and form is vastly superior to Fiji control seedlots, e.g. approximately 50% greater wood production. The estimated improvement in volume yield from using better seed sources is 4 m^3 ha^{-1} yr^{-1}. The stumpage paid for better-class mahogany logs is about $US 58 per m^3 and sawn timber recovery is about 50% (45-60% depending on saw type and log size/grade). If the mahogany plantation estate were progressively re-planted using superior seed sources the returns to Fijian landowners would an extra $US 9.3 million, and additional annual export revenue return to the Fijian economy of about $US 93 million. However, the main replanting is being done by the Fiji Hardwood Corporation (FHC) and this corporation is only now becoming aware of the importance of using the best seed sources.

The Fiji Forestry Department/SPRIG are planning to establish a grafted clonal seed orchard of the best individuals/families which will provide improved seed in about 8-10 years. The long time to fruiting might be able to be reduced through intensive management and use of paclobutrazol. In the meantime there would be substantial economic benefits to Fiji mahogany growers from using imported seed of the identified superior sources.

Santalum **(sandalwood)**

Santalum yasi produces a highly prized sandalwoods, similar in quality with the well-known *S. album* from India and Indonesia. The heartwood of *S. yasi* was a major export during the early 19th century and the sandalwood trade was one of the first attractions drawing Europeans into the South Pacific. Sandalwood from *S. yasi* is still exported to a limited extent from Fiji and Tonga, experiencing short-lived boom periods associated with a buildup of sandalwood stocks, the most recent in Fiji being in the mid-late 1980's when a ban on commercial exploitation was lifted.

The primary advantages of growing sandalwoods are their ability to produce a very high-value, non-perishable product (heartwood) which can provide cash income to people living in outer islands and more remote communities (Thomson 2006). Surprisingly, up until very recently, there has been limited planting of sandalwood in the South Pacific. This has been largely due to lack of seedlings and a poor understanding of silvicultural requirements resulting in poor survival and slow growth. Over the past 5 years, there has been an increased interest in sandalwood planting in Vanuatu, Fiji, Tonga and Cook Islands: most of the planting has been by smallholders and in home gardens. The largest planted areas would generally not exceed several hectares. The Fiji Forestry Department has found that F1 hybrids between the local *S. yasi* and the introduced *S. album* grow about 300% faster than yasi and 32% faster than album. The hybrids also have better survival than the parents and begin forming the valuable heartwood from an early age, e.g. about 6-7 years. SPRIG and it national partners are pioneering technologies, including potted grafted orchards, which will lead to the rapid production of sandalwood hybrid germplasm, with substantial seed supplies becoming available within 1 to 2 years.

It is reasonably expected that the supply of improved germplasm/seedlings and grower information will lead to the annual production and cultivation of tens of thousands of more plants of sandalwood in each country (Vanuatu, Fiji, Tonga and Cook Islands[3]). Under fertile conditions a suitable rotation age for hybrid sandalwood is anticipated to be in the range 20 to 25 years. However, given good silviculture including regular weeding in early years and interplanting with a mixture of compatible long-term hosts, the rotation age for hybrid sandalwood might be reduced to 18 to 20 years at which time each tree might have produced 40-80 kg of heartwood, valued at $US 900 to 1,800. Therefore hybrid sandalwood plantations (planted at recommended spacing of about 250 trees per hectare, with 50% survival) can conservatively provide $US 112,500 - 225,000 at harvest time. Individual small trees grown in home gardens can provide up to $US 8,000 each on longer rotations (40-50 years).

***Terminalia catappa* (tropical almond)**

Whilst *Terminalia catappa* is widely planted throughout the humid tropics for coastal protection and provision of shade its possibilities as a fast-growing multipurpose tree have yet to be tapped (Thomson and Evans 2006). The main commercial products of tropical almond are sawn timber for local use, especially in house and building construction, bark for traditional medicines and nuts for human consumption. The trees are also important for food security, having good cyclone resistance and bearing nut crop within 3-4 months of major cyclones.

The work of SPRIG and partners on *T. catappa* has been mainly to identify populations and trees which have had their nut characteristics improved through traditional selection methods, including specific localities/islands in PNG, Solomon Islands and Vanuatu. The aim is to make these improved materials more widely available in the South Pacific. This process has already started to take place in Solomon Islands with improved varieties introduced from Santa Cruz to Western Province, in Vanuatu with better nut phenotypes from different parts of Vanuatu planted into seed orchard on Santo, and now producing seed/seedlings for extension and in Tonga with improved nut types introduced from PNG and Solomon Islands and planted on ‘Eua, Tongatapu, Ha’apai and Vava’u islands.

In Vanuatu, a local company (Kava store) purchases sun-dried kernels-in-testa for about $US 8 per kg from a network of suppliers around the country. The supply of nuts is insufficient to meet domestic urban demand because there are not enough trees and/or suppliers, trees are too scattered and yields too variable.

The economic gains from replanting with better nut-types of *T. catappa* are equivalent to about \$50 – 100 per tree (once trees come into heavy production, i.e. after about 4-6 years). The number of superior nut trees planted to date through SPRIG partners is several thousand, and this is expected to expand to several thousand per year over the next 5-10 years. Accordingly the economic benefits from planting of selected nut types of *T. catappa* are predicted to be significant in each country (e.g. > \$50-100,000 per year).

Tectona grandis **(teak)**

Teak is one of the world's most-sought after hardwood timbers, especially for furniture and boat building. Sawn boards of good quality plantation teak typically sell from more than \$US 1,600 m^3 in the international market place.

Improvement of teak in Solomon Islands (SI) has gained momentum through both phases of SPRIG. In Phase 1, teak seed was collected from different regions and imported from overseas and used for a progeny test (and for future improvement). In late 2003 an agreed working plan for teak improvement was developed by SPRIG/CSIRO with and for the two major commercial growers (Kolombangara Forest Products Ltd and Eagon Pacific Plantations Ltd) and the SI Forestry Division. A new teak seed stand will be established using further selection of new material from the provenance trial established during SPRIG 1. This is necessary to support supply of high quality genetic material to the rapidly expanding smallholder/family-based reforestation program.

In 1992 an excellent local teak seed source, but of limited seed production capacity, has been established in SI through grafting of about 50 selected trees. The plants from this seed source have outstanding stem form and vigor (estimated to have > 30% greater volume increment than other sources). SPRIG has provided equipment and training to enable teak seed to be safely collected from this and other local sources. The area of teak in SI was 1439 ha in 2002 and it is estimated that > 500 ha of new teak is being established each year by private tree growers. The gains to smallholders from planting improved teak are estimated to be 3 m^3 ha^{-1} yr^{-1} (increased productivity) x \$US 120/m^3 (price to growers) or \$A 360/ha. For an teak plantation resource of 5,000 ha (based on improved teak) this equals an additional \$US 1.8 million per annum to growers. The extra export income to SI is estimated to be around \$US 7.9 million per annum (calculated as 5,000 ha x 3 m^3 ha^{-1} x 0.35 (sawn timber recovery) x \$US 1,500/m3). This is a conservative estimate as it does not take into account the superior form of the improved teak, and associated higher recovery rates.

Pandanus tectorius (**Pandanus*****)***

Pandanus (*P. tectorius*) is a large shrub or small tree of immense cultural, health, and economic importance in the Pacific Islands, second only to coconut in terms of importance for atoll communities (Thomson *et al.* 2006). A highly variable species complex, it grows wild mainly in semi-natural vegetation in littoral habitats throughout the tropical and subtropical Pacific, where it can withstand drought, strong winds and salt spray. Hundreds of traditional varieties of *P. tectorius* have been recognized, named and propagated by local people in different parts of the Pacific islands. Vitamin A deficiency is a major cause of blindness, ill health and death in the northern Pacific. Increased exploration, conservation, replanting and consumption of local food crops rich in Vitamin A provides an effective process for mitigating this major health hazard.

The impetus for increased research and conservation of *Pandanus* varieties in the northern Pacific stems from proposals in 2000 from Terimawa Natake (Ministry of Environment, Lands and Agricultural Development in Kiribati) and Nancy Van de Velde (Ministry of Resource Development and Works, Marshall Islands) to SPRIG. This work has included documentation of traditional knowledge, especially on varietal uses and characteristics, collection, characterization and establishment of a varietal field gene bank on South Tarawa.

Recently a pandanus cultivar which has the highest level of beta-carotene found so far (beta-carotene is the carotenoid with the most vitamin A activity) (Englberger *at al.* 2006). This is Te Arabukitaba at 896 micrograms, much higher than the cultivar found in the last set with the highest level, that being Te Kaureiko at 410 micrograms. This shows the importance of continuing the investigation of the different pandanus cultivars so that a better understanding of their potential health benefits is gained. This work is continuing through a collaboration of SPC/FAO/USP and national partners and the Island Food Community of Pohnpei.

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Comparative Sequence Analysis of the Internal Transcribed Spacer 2 of *Pinus brutia* and *Pinus halepensis* Populations from Turkey

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Abstract

Turkish red pine (*Pinus brutia*) is wide-spread and an important forest tree species in Turkey, occurring mainly in southern, western and north-western Turkey and in small isolated populations within the areas in the Black Sea region. Aleppo pine (*Pinus halepensis)* has naturally found only in Adana and Muğla provinces as small population in mixture with Turkish red pine. Although Turkish red pine and Aleppo pine are morphologycally different, Turkish red pine has been regarded by some taxonomists as subspecies of Aleppo pine due to occurrence of natural hybridization between these two species. However, the phylogenic relationship between these species needs to be explored further. In the present study,by sampling overlapped populations of both species from Muğla and Adana provinces (3 populations of Turkish red pine and 2 populations of Aleppo pine), ITS region of ribosomal DNA were comparatively studied with sequence analysis. Although ITS1,5.8s and ITS-2 regions were studied with ITS primers designed by Rogers and Kaya (2006), only ITS-2 region was successfully amplified with PCR. The complete data set for this region was analysed using MEGA3.1 software. ITS amplicons from PCR amplification with ITS-2 primers were about 420bp long. There are high sequence homology between Turkish red pine and Aleppo pine populations of Turkey. There are only a few variable and parsimony informative sites at the individual/population level. Observed branching patterns of Turkish red pine and Aleppo pine in phylogenic tree constructed with ITS-2 sequence data were weakly supported by bootstrap analysis (below %50). Based on the results of ITS-2 region sequence analysis, Turkish populations of Aleppo pine and Turkish red pine populations could not be fully differentiated. Since ITS-2 region of nuclear ribosomal DNA revealed a few variable and parsimony informative sites for both species, thus, ITS-2 region of ribosomal DNA appears to be inadequate for resolving genetic relationships between Turkish red pine and Aleppo pine populations in Turkey. Further studies dealing with ITS-1 and 5.8s of ribosomal DNA and populations included from major Aleppo pine distribution areas will be useful to understand the evolutionary relationship between Aleppo pine and Turkish red pine populations in Turkey.

Keywords: *Pinus brutia*, *Pinus halepensis*, ITS region, Phylogeny

Inheritance of Wood Specific Gravity and Its Genetic Correlation With Growth Traits in Young *Pinus brutia* Progenies

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Abstract

The demand for wood products can not be met with the increased population growth in Turkey. The gap between wood supply and demand is over 2 million m^3 in Turkey at the moment. The studies carried out in recent years have indicated that tree improvement is the most effective and least costly approach to increase wood productivity and quality. Turkish red pine (*Pinus brutia* Ten.) is one of the most important forest tree species of Turkey and covers 21 % of total forest areas in the country. Because of large distribution area, early sexual maturity and existence of high genetic diversity as well as high wood specific gravity values, Turkish red pine is considered by Turkish foresters as a highly valuable species among the native tree species of the country. That is why, the species is determined as the priority tree species included into the *Turkish National Tree Breeding and Seed Production Program*. The goal of Turkish red pine tree improvement program is to improve both growth and wood quality traits since wood specific gravity (WSG) is the most important trait for evaluation of pulps for paper mills, furniture, and lumber industry and it is strongly correlated with wood quality traits.

With the present study, the determination of wood specific gravity and its inheritance in Turkish red pine, potential genetic gain with early selection of trees for WSG were aimed to provide basic information for effective tree breeding and guidance in the Turkish red pine Tree Breeding Program which is still at early stage of breeding program. For this purpose, the wood samples for determining WSG were obtained by destructive sampling from the Turkish red pine progeny tests established with 168 half sib families in Ceyhan Forest Nursery located in the eastern Mediterranean Region of Turkey. The wood samples were taken from 1.30 m above ground parts of stems of trees which were removed during the thinning program and WSG of samples were determined according to Kaya *et al.* (2002). For WSG, heritability, genetic gain and other parameters for effective breeding of Turkish red pine were estimated by genetic and statistical evaluation of data.

\The mean WSG was 0.432 and ranged from 0.351 to 0.571 (wood density range: 351-571 kg/m^3). Estimated genetic gain was calculated 10 % based on selection of 20 plus trees with the highest WSG. Individual and family heritabilities for wood density were 0.39±0.07 and 0.55±0.05, respectively. These results indicated that wood specific gravity is under strong genetic control and family selection is an effective way to obtain genetic gain in short terms. Although there was slightly negative genetic correlation between wood density and height at age 4 (r_g=-0.21) however, there was no genetic correlation between wood density and height growth at age 7 ($r_g=-0.03$) as well as between stem volume at age 7 and wood density ($r_g=0.01$). These genetic parameters for WSG in *Pinus brutia* should encourage the Turkish Forest Trees and Seed Breeding Research Directorate to practice early selection for this trait for *low elevation breeding zone* .

Keywords: Pinus brutia, specific gravity, early selection, heritability, genetic correlation

Primary Selection of Superior Clones of *Populus davidiana* Based on Disease Resistance and Growth Performance on a Nursery Bed

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Abstract

We have investigated the severity of poplar leaf rust and leaf spot and growth performance of the progenies of 40 combinations of *P. davidiana* Dode to select and propagate superior clones. The severity of poplar leaf rust based on the Schreiner scale varied among combinations. A 52.5% of 40 combinations were susceptible showing that they were all '100' (the most susceptible clone scoring) on average severity rate. The progeny of a combination of Bangdong1 x Canada1 was the most resistance to leaf rust (average severity rate was 3.8). The severity of leaf rust was positively correlated with the severity of leaf spot (r=0.46; p<0.01). There was no correlation between the severity of leaf rust and height (r=0.01; p=0.93), but the severity of leaf rust and diameter at root collar was negatively correlated (r=-0.34; p=0.03). The severity of leaf spot was negatively correlated with height and the diameter at root collar (r=-0.39; p<0.05; r=-0.59, p<0.001, respectively). Superior clones of 22 individuals were selected based on their scores on disease resistance to leaf rust and leaf spot and growth performance.

Key words: leaf rust, leaf spot, Schreiner scale, severity rate, diameter at root collar

Introduction

Aspen is belonging to Treppidae, sub-section of section Leuce that is one of the six sections of *Populus*. Breeding for aspen was practically started in northern Europe from 1940s' when crossing between *P. tremula* and *P. tremuloides* was initiated for commercial purpose. In Korea, aspen breeding was started from late 1980s'. Noh et al. (1992) established the guideline of rotation age and the early selection age of aspen. Noh et al. (1997) and Koo et al. (2001) conducted the selection of superior aspen clone. Poplar leaf rust caused by *Melampsora* can be damaging to poplar (Wang and van der Kamp, 1992), and even be related to the mortality of young poplars (Newcombe et al., 1994). Cercospora leaf spot caused by the fungus, *Cercospora populina*, continues to be a devastating foliar disease of *P. davidiana* in Korea. Cercospora leaf spot occurs from early July to the late autumn, and infected leaves fall down rapidly from early August. This study was conducted to select and propagate superior clones based on disease resistance and early growth performance.

Materials and methods

In the natural aspen stands, 258 aspen clones were selected based on their growth performance throughout the nation. These selected clones were planted at seven regions in 1991(25 clones) and 1992(40 clones) to evaluate the growth performance. In 2002, from the clones showing excellent growth performance, branches for artificial crossing were collected. Pollens from male flowers were used to make 40 artificial cross combinations of *P. davidiana*. Seeds from the each combination were planted in pot and transplanted on a nursery bed when they were 10-15cm in height. Rust severity was rated on September 30 and October 2, 2003, using the Schreiner (1959) scale (0-100). The most severely rusted leaves in a tree were estimated as light, medium, or heavy and given a numerical value of 1, 5, or 25, respectively. The percentage of leaves rusted was estimated at ≤25%, 26-50%, 51-75%, or >75% and given a numerical value of 1, 2, 3, or 4, respectively. Then multiplication of the two numerical values gave a Schreiner rating from 0 (highly resistant) to 100 (highly susceptible). The severity of the Cercospora leaf spot was rated in the ratio of damaged area against total leaf area. The percentage of leaves damaged was estimated at <10%, 10-24%, 25-49%, 50-75%, or >75% and given a numerical value of 1, 2, 3, 4, or 5, respectively. Height and diameter at root collar were measured with randomly selected fifty individuals from each combination. Survival rate was also investigated in October 2003.

To determine differences in growth performance among combinations, data were analyzed by analysis of variance using the SAS-PROC GLM statistical package and type III sums of squares. The exploratory PROC univariate test was done on all variables to determine whether they satisfied the assumption of a normal distribution. To compare the relationship between growth performance and severity rate of the disease, Spearman correlation coefficient was obtained using SAS-PROC CORR.

Results

The severity of poplar leaf rust based on the Schreiner scale varied among combinations. Average severity of the leaf rust of 40 cross combinations was 63.2. A 52.5% of 40 combinations were susceptible showing that they were all '100' (the most susceptible clone scoring) on average severity rate. The progeny of a combination of Bangdong1 x Canada1 was the most resistant to leaf rust (average severity rate was 3.8). The severity of leaf rust was positively correlated with the severity of leaf spot ($r=0.46$; $p<0.01$). There was no correlation between the severity of leaf rust and height ($r=0.01$; $p=0.93$), but the severity of leaf rust and diameter at root collar was negatively correlated ($r=-0.34$; $p=0.03$). The severity of leaf spot was negatively correlated with height and the diameter at root collar ($r=-0.39$; $p<0.05$; $r=-0.59$, $p<0.001$, respectively). The average severity of Cercospora leaf spot of 40 cross combinations was 3.11. The progeny of Ohdae19 x Wundoo2 combination was the most resistant (average severity rate was 2.13) to the leaf spot, however, the progeny of Jungma4 x Palgong3 combination was the most susceptible (average severity rate was 3.77) to the leaf spot. Average survival rate of 40 cross combinations two years after transplanting in nursery bed was 52.7%. Ohdae19 x Wundoo2 showed the highest survival rate (93.9%) and Ohdae19 x Palgong3 showed the lowest survival rate (15.5%).

Conclusions

The average severity of poplar leaf rust of the two-year-old progenies of 40 cross combinations of

P. davidiana was 63.2 by the Schreiner rust rating. A 52.5% of 40 combinations were highly susceptible to the leaf rust. The progeny of a combination of Bangdong1 x Canada1 was the most resistant to leaf rust. The severity of leaf rust was correlated with the severity of Cercospora leaf spot and survival rate. No correlation was shown between the severity of leaf rust and height. The severity of leaf spot was negatively correlated with height and the diameter at root collar. Statistically significant differences were found among 40 combinations in height and diameter at root collar. The progeny of a combination of Ohdae19 x Wundoo2 was the tallest (2.46m±0.4m), and the progeny of Bangdong1 x Canada1 was the poorest in height (1.4m±0.41m) and diameter at root collar (0.9cm±0.37cm). Nine combinations that show less than 20 in the Schreiner scale, less than 3.1 (average) in Cercospora leaf spot rating, and better than average in height and diameter at root collar were selected. Among the combinations, superior 22 individuals showing good growth performance and traits were selected for future test.

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Supporting Organizations

International Union of Forest Research Organizations

Akdeniz University, Antalya, Turkey

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