

Seed Orchards and Breeding Theory Conference

21 - 25 May 2012
Antalya, Turkey

Proceedings

Editor

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Seed Orchards and Breeding Theory Conference

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Preface

The international conference in Turkey will share and discuss recent developments and experiences on seed orchards, improved seed materials and its using in forestry, including breeding theory, which supports the production of improved material. Seed orchards are a most important link between forest breeding and practical forestry, and serves a better future for mankind. Important aspects are sustainable forestry considering environmental changes and problems like global warming. Seed orchards can improve unproductive land increasing its ecological value and contribute to economically and biologically successful plantation programs. In many programs seed orchards appear very profitable. A society investing in forest tree breeding and seed orchards demonstrates a will to invest for the future, to think long term and do something for future inhabitants of the area the society is responsible for.

The conference is the third in a row of seed orchard conferences, earlier took place at Umeå 2007 and Korea 2009. The conference is a part of the activities of IUFRO WP 02.09.01 seed orchards, WP 2.02.13 breeding and genetic resources of Mediterranean conifers, and WP 2.04.02 breeding theory and progeny testing.

This proceeding contain abstracts of planned presentations, 44 oral and 8 poster presentations, from 19 countries and five continents. Full versions of the presentations are planned to be available in three months after the conference.

Thanks to all invited speakers, moderators, contributing speakers, poster presenters, and conference participants for their contributions as well as sponsors and committee members for their strong support to the meeting. And also thanks to Managements of Suleyman Demirel University for their administrative and financial support.

Nebi Bilir,

Conference Secretary, Proceedings Editor

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ORAL PRESENTATIONS



Breeding Population of *Quercus glauca* for a Seed Orchard Regarding the Climatic Factors

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Abstract: Seedlings of *Quercus glauca* from naturally crossed seeds of 35 selected families growing in Jeju Island were placed using stroll repeated planting into two experimental forests located at Hannam and Sanghyo in Jeju. After 3 years of planting, characteristics of growth were examined. Growth pattern of trees in Sanghyo was better than those in Hannam. A great weather differences especially in precipitation and temperature were shown between two test sites. The result of difference within families and individuals obtained from this study was also useful for establishment of seed orchard of *Q. glauca*.

Key words: Experimental forest, *Quercus glauca*, Growth pattern, Native plant, Family, Weather factor.

Objectives : This study was done to establish the growth strategy; a breeding population of *Q. glauca* has been built *via* simple recurrent selection among native stands grown in Jeju Islands in southern part of Korean peninsula.

Introduction and Background: A wide variation among forest ecosystem is observed with respect to the climatic factors such as temperature, amount of precipitation and light condition. According to global warming, the forest ecosystem tends to move toward from south to north. Ecologists and forest researchers are looking to see the phenomenon of forest ecosystem reaction. Trees growing in a certain ecosystem could show a response as the morphological characteristics, e.g., height, diameter, leaf size and branching pattern, which can be interpreted in

terms of the different growth strategies. Among these trees, a certain species expresses a special sensitive growth pattern. The tree of *Quercus glauca* is sensitive to climate change. Also it could be an indicator for the global warming occurring in Korean Peninsula.

Methods: Among the native populations in Jeju Island, outstanding phenotypic mother trees have been directly selected and 35 families have been chosen from the year of 2002 to 2004. Seeds were collected from the selected trees and seedlings were produced from the naturally crossed seeds of these selected trees. In 2006, the seedlings were planted using stroll repeated planting method into two experimental forests located at Hannam and Sanghyo in Jeju. After 3 years of planting, characteristics of growth such as height and diameter of root collar were examined. All climate factors of year from 2006 to 2009 were serviced by Seogwipo meteorological office in Jeju Island. Among the climatic factors, data about the amount of sunshine, warmth index (temperature $>5^{\circ}\text{C}$) and periodic precipitation which could mainly affect on the growth of seedlings were analyzed.

Results

Growth comparison at two test sites: According to the t-test, plantlets in Sanghyo were show better growth. The average height of plantelets in Sanghyo was 92.6 cm while it was 66.8 in Hannam. And the average root collar diameter was 12.2 mm and 9.3 mm in Hannam (Tables 1 and 2).

Table 1. Growth characteristics (height) of *Q. glauca* planted at two sites.

Site	N	Mean (cm)	Std. Dev	Std. Err
Hannam	1219	66.8302	30.0853	0.8617
Sanghyo	1153	92.523	37.7482	1.1117

Table 2. Growth characteristics (root collar diameter) of *Q. glauca* planted at two sites.

Site	N	Mean* (mm)	Std. Dev	Std. Err
Hannam	1219	9.2568	2.9787	0.0853
Sanghyo	1153	12.2151	6.7895	0.2

*Root Collar Diameter.

Climatic factors: The periodic precipitation was much more in Hannam sites. It was almost 3,000 mm in Hannam while it was less than 2,000mm in Sanghyo (Table 3). Also the monthly temperature was higher in Sanghyo site than in Hannam site. These differences could be used for helpful information to establish a certain breeding population including seed orchard (Figure 1).

Table 3. Amount of periodic precipitation (mm) in two planting sites of *Quercus glauca*.

Period	Seogwipo	Hannam
2007.06.12	1,724.8	3,271.3
2008.01.12	1,661.4	2,911.0
2009.01.09	1,726.2	3,191.0

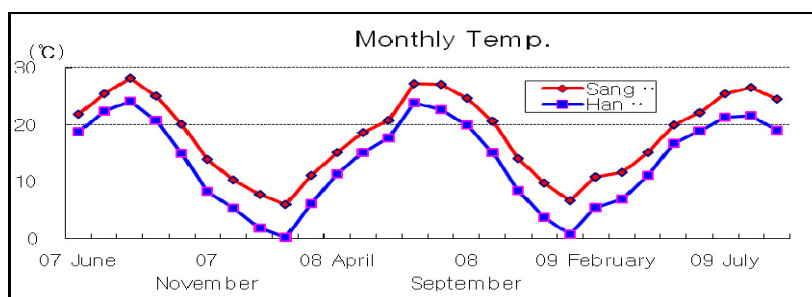


Fig. 1. Monthly change of temperature in two planting sites of *Quercus glauca*.

Best clones both in two test sites: Among all 35 families tested in this study, only two families of No. 12 and 14 were show good growth both in Hannam and Sanghyo (Table 4). These two best families should be propagated as super clones to acquire more genetic gain. Also the result of difference of growth pattern between two sites could be useful in provenance adaptability test.

Table 4. Height and RCD of two families of *Q. glauca* which grow better in both Sanghyo and Hannam.

Family No.	Height (cm)		R.C.D* (mm)	
	Sanghyo	Hannam	Sanghyo	Hannam
12	103.9	80.8	13.4	12.8
14	114.5	81.1	13.1	8.8

*Root Collar Diameter.



Evaluation of Spontaneous F₁ Inter-specific Hybrids of *Eucalyptus citriodora* and *E. torelliana* Planted at Different Locations

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Abstract: Eucalypts being a predominantly cross-pollinated species can be easily improved through hybridization. Hybridization also offers the scope to capture the benefits of hybrid vigour which is sometimes expressed in certain specific species combinations. Controlled hybridization is very cumbersome and labour intensive. At Forest Research Institute, Dehradun in a field trial, the parent species of *E. citriodora* and *E. torelliana* have been planted in 1987. Both the species falls under the sub-genus corymbose. These species have been planted side by side in line at 2m x 2m spacing. The open-pollinated seeds from *E. citriodora* and *E. torelliana* have been collected, progenies raised and inter-specific reciprocal natural hybrids of *E. citriodora* and *E. torelliana* picked up. The natural hybrids have been identified through morphological genetic markers. The percentage of hybrids of *E. citriodora* x *E. torelliana* varies from 6-8% and *E. torelliana* x *E. citriodora* 1-2%. Some of the hybrids exhibited high degree of hybrid vigour even at the nursery stage which sustained even at the later stage of their development. These hybrids along with parents have been planted in the field in the year 2007. Their growth performance of hybrids and parents in the field is reported here.

Key words: *Eucalyptus*, cross-pollinated, hybrid vigour, *E. citriodora*, *E. torelliana*, morphological genetic markers.



Management of Seed Orchards through Estimation of GCA and Breeding Values from a F_2 Progeny Trial and Forward Selection in *Eucalyptus camaldulensis* Dehnh.

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Introduction: Cropping systems in forestry is becoming more planned systems in recent years through genetically improved crops and facilitate improving the productivity in terms of wood production and quality. In this process, progeny trials are increasingly becoming important to test the fidelity of gains achieved in the parent clones and also to direct variation toward some particular outcome for further exploration in next generation's search space (Goldberg *et. al.* 1993; Sumita and Sindhu, 2011). When selection in half-sibs are attempted in a progeny trial, the General Combining Ability (GCA) and breeding values obtained from best linear unbiased prediction is important criteria for selection of genotypes, because GCA is the outcome of interaction between alleles at gene loci and represents an average performance of the progeny of an individual when it is mated to a number of individuals in an orchard (Zobel and Talbert, 1984; Xie *et. al.* 2007). Through breeding it is possible to manage inbreeding in the populations and seed orchard can be managed by thinning inferior trees. Such seed orchards are likely to serve as a gene package with genetically improved seed at the end of a generation of breeding. Therefore forward selection based on GCA and breeding values is more promising to increase the genetic gains. A large number of parents may be selected through this method compare to selection through average index score method (White and Hodge, 1988).

Key words: breeding, genetic gains, out-crossing, inbreeding, panmixis.

Material and method: An *Eucalyptus camaldulensis* F_2 half-sib progeny test with 49 families was established during 2002 for evaluation of their performance for fast growth and also for inclusion into F_3 generation breeding program. The parents of

progenies were selected from one seedling seed production area and two seedling seed orchards. The plants were planted in RCBD with four replications with seven seedlings assigned randomly to each replication and planted in contiguous plots. Mortality rate was 4.7% at age 2 years. Growth components such as total height, clear bole height and girth at collar were recorded and analyzed. Based on the performance of progenies, GCA and breeding values of mother trees were estimated and the implications of results in the management of seed orchards were discussed in this communication.

Discussion: Total height, clear bole height and girth at collar exhibited highly significant intra-genotypic and population variation (all components $p = < 0.001$) suggesting the canalization of growth factors from parents to progenies and indicate the heritable nature of those characters. Higher degree of canalization results in higher phenotypic variability (Waddington, 1960) and suggested the underlying differences between components in the mechanisms responsible for character expression or differences in the efficiency of the buffering mechanisms themselves, thus creating well balanced genotypes with better buffering against environmental perturbations (Clarke, 1998).

Linearity of the growth characters were reported by many workers and strong positive relationship among total height and diameter was established in trees. Good GCA and breeding values for all three growth components were observed in tree no. 57, 67, 90, 69, 88 and 89. While tree no. 71 and 74 had good GCA and breeding values for total height and clear bole height, tree no. 59 and 94 have good values for both total height and girth indicating correlated growth traits which facilitate simultaneous improvement of multiple traits and such correlated growth traits were reported for both height and mean annual increment in *E. grandis* (Osorio *et. al.* 2003).

Breeding values reflect the interaction of alleles at particular loci when mated with a number of individuals within a population. If families suffer from selfing and mating with near relatives may result in differential family performance which lead to significant differences in breeding values in open-pollinated progeny trials like the one we have studied. Comparison of growth rates of self-pollinated and cross-pollinated families demonstrated negative effects of inbreeding in seed viability and growth in *E. globulus* ssp. *globulus* and positive association between out-crossing rate and growth in *E. grandis* (Burgess *et. al.* 1996). Besides, significant positive differences were reported in growth and survival and also in height and diameter in the families of two year old trees in a progeny trial of *E. camaldulensis* due to

differential rate of out-crossing rates (Butcher and Williams, 2002). Therefore, it may be assumed that genotypes with good out-crossing rates have wide positive differential and better heritability rates in height and diameter. Out-crossing rates accounted for about 13 % and 23% of variation among families within populations in height and diameter respectively (Butcher and Williams, 2002) and the occurrence of low level of variation explained by differences in out-crossing rates does not warrant adjustment in the breeding program of *E. camaldulensis*.

There were reports that eucalypts were suffering from moderate inbreeding (Brown *et. al.* 1975) and the genetic gains in yield may be reduced from recurrent selection in open pollinated seed orchards due to new combination of genotypes on which feature genetics gains by selection depends. Hence, the wide differences in families breeding values suggested for forward selection of genotypes since the differences in genetic gains achieved from recurrent selection may be overcome by benefits of out- crossing within the population (Xie *et. al.* 2007). Meanwhile, four parents from SSO of Sathyamangala and one from SSPA of Pondicherry were the worst performers in GCA suggesting narrow selection range or family co-ancestry in those parents and they can be culled to avoid dilution of panmixis (Soh 1994). With increase in intensity of selection, variations remained significantly large and have a tendency to increase due to environmental perturbations. Hence, 10 best genotypes with high breeding values for each growth component were selected for synthesizing F₃ generation breeding population.

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Analyses of Growth and Wood Parameters for Quality Seed Production in Clonal Seed Orchard of *Dalbergia sissoo* Roxb.

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Abstract: *Dalbergia sissoo* Roxb. is known for production of quality timber worldwide from ancient times beside other uses. The Indian native though has been an economically important species in making priced furniture, panels and handicrafts, little attention is given towards its genetic improvement particularly for the improvement of its poor productivity, crooked stem and die-back disease, which leads to greater loses both in plantations and workshops. During 1996-97, therefore a clonal seed orchard (CSO) consisting 27 genetically divergent clones, selected from the distribution range of species from Northern India and Nepal, was established at Pindori Mindo Mind, Punjab, India (latitude 31°31'36"N and longitude 75°48'54"E). The CSO was enumerated for growth characters to develop index values for individual ramets as well as clones. A total of 12 clones with maximum index value were selected for further analysis of wood parameters and heartwood formation. The core samples of the wood were obtained through increment borer at the breast height (1.37 m) to analyze important wood parameters including wall thickness, vessel element length, vessel element diameter, fibre length and specific gravity. Divergence analysis based on Mahalanobis D^2 statistics, which calculated degree of diversification and relative proportion of each component character to the total divergence among total individuals, was studied to measure differentiation at intra and inter cluster levels resulted in grouping selected clones into seven clusters. Cluster I and II contained four (clones 41, 192, 33 and 57) and three clones (Clone 2, 19 and 12) respectively, and remaining clusters had just one clone each. The clone originated from Barielly, Uttar Pradesh of cluster VII was

found to be the most divergent clone. Cluster II maintained greater inter-cluster distance with other clusters. The intra-and inter cluster distance ranged from 0.00 to 4.06 and from 5.35 to 74.44 to indicate ample diversity among clones. It was obvious that the clones selected from a single region were spread over different clusters, which was probably due to high degree of heterozygosity of different genotypes, genetic architect, higher intensity of selection and high degree of general combining ability. The divergence analyses confirmed that the clones planted in the clonal seed orchard were sufficiently divergent and have sufficient opportunities of natural hybridization to produce quality seed. The seed harvested from this orchard has been maintaining high genetic diversity and used for the establishment of commercial plantations.

Key words: *Dalbergia sissoo* Roxb., clonal seed orchard, wood and growth traits, genetic diversity, hybridization.



Production of Improved Seed from Advance Generation Seedling Seed Orchard of *Dalbergia sissoo* Roxb.

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Abstract: A seedling seed orchard (SSO) of *Dalbergia sissoo* Roxb. consisting thirty four most promising progenies was established during 1997-98 at Mettiwara, Punjab, India ($75^{\circ}59'11.4''E$ and $30^{\circ}50'08.3''N$) under hot and semi-arid conditions. The SSO was established in completely randomized block design with six replications and five plants in a row. After completion of 10 years of age, a mid-term assessment was carried out during 2007-08 through index method adopted with five traits viz. diameter at breast height (DBH), height, clear bole height (CBH), stem straightness and general health. Every progeny of the SSO, after thorough enumeration and evaluation, was roughed by about 50 % basically to enhance inter-breeding among genetically divergent genotypes / progenies with synchronized flowering to exploit maximum heterosis resulted due to natural hybridization. The selection differential of the roughed SSO was recorded to 2.25, 1.48, 0.51, 0.60, and 0.64 for DBH, Height, CBH, Straightness and Health, respectively. Further, height and DBH were analyzed for genetic parameters both in base and retained populations. The heritability could be improved substantially from 0.0525 to 0.3008 for height and 0.1186 to 0.3843 for DBH in base and retained populations, respectively. Similarly, the genetic advance improved from 2.43 to 5.14 and from 9.29 to 11.68 respectively for height and DBH with net genetic gain of 10.20 and 13.47 %. It could therefore well be concluded that the seed collected from the roughed SSO would sustainable enhance the quality of future plantations established through seed of this seed orchards and increase net income of the growers.

Key words: *Dalbergia sissoo* Roxb., seedling seed orchard, heterosis, heritability, genetic advance, genetic gain.



Gametic and Genetic Contribution in a Clonal Seed Orchard of *Pinus tabuliformis* Carr.

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Abstract: *Pinus tabuliformis*, also named Chinese pine, is an important native coniferous tree species to northern China. It is naturally distributed across 14 northern provinces and autonomous regions, with a land cover of close to 3 million km². Since 1970s, genetic improvement of Chinese pine as indigenous conifer tree species in northern China was started and followed by clonal seed orchard establishment. In this paper, we investigated the clonal reproductive success and synchrony, quantity of staminate and ovulate strobilus and the average number of full seeds among the selected 49 clones in a clonal seed orchard located in Xingcheng City (Liaoning Province, China) from the initial seed harvest to the stable seed production over a 12 years span. Combined with results from 3 seedlots open pollinated progeny test of the seed orchard, the clonal gametic and genetic contributions to the seedlots of the seed orchard were estimated and factors related to the clonal genetic contribution were also calculated. Results indicated that the reproductive synchrony index of pollen shedding has shown to be higher than that of the strobili receptivity, and both can be drastically influenced by the ambient climate factors. Reproductive synchrony index of the clones has certain relative stability and it could be used as an indication of the seed orchard status during maturity stage; clones in the studied orchard have shown extreme differences in terms of the gametic and genetic contribution to the seed crop at the orchard's early production phase specifically when they severed as either female or male parents and those differences were closely related to clonal sex tendency at the time of orchard's initial reproduction. Clonal gamete contribution as male and

female parent often has a negative correlation, utilization of a clone as pollen, seed or pollen and seed donors should consider the role it should play in the seed crop; Due to numerous factors influencing on the mating system in seed orchards, clonal genetic contribution as pollen parent was uncertain, and it has major influence on the genetic composition in the seed orchard during the initial reproductive and seed production phase. According to correlation analyses, clonal flowering synchronization, clonal gamete contribution, quantity of staminate and ovulate strobilus, and number of full seeds per cone were the most important factors influencing the genetic contribution of each clone. Results of this paper are expected to provide the theoretical foundation for seed orchard management, genetic composition prediction and advanced seed orchard establishment of the Chinese pine.

Key words: Chinese pine; Gametic contribution; Genetic contribution; Clonal seed orchard; Breeding value.



Assessment of Fertility Variation in Three South Indian Forest Tree Species

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Abstract: Fertility variation was estimated in three tropical forest tree species (*Azadirachta indica*, *Tamarindus indica*, and *Santalum album*) for two consecutive years in southern India. Fertility variation was described as sibling coefficient (ψ), estimated based on flowering abundance and seed set in four year old plantations. Fertility varied between trees within populations and between successive years in all the species. Male and female fertility variation differed substantially between years in *S. album* compared to *A. indica* and *T. indica* populations. Fertility variation is usually high during poor flowering years and in young populations as evident in all three species. The combined sibling coefficient value in the studied populations ranged from 3 to 4.4, though the male and female fertility differences were high in *S. album*.

Key words: Tropical forest, tree population, fertility, flowering, seed set, sibling coefficient.



Fertility Variation and Effective Population Size in Seedling Seed Orchards of *Acacia auriculiformis* at Three Diverse Sites in Southern India

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Abstract: Seedling seed orchards of *Acacia auriculiformis*, were established at four diverse locations (SSO I - IV) in southern India using bulk seed collections from natural provenances of Australia. The seedling orchards were established using the best natural provenances identified for growth and stem form.

The orchards were evaluated for fecundity, fertility variation and effective population size at five and six years of age. Fecundity was influenced by total annual rainfall, decreasing from SSO I (receiving 1810 mm rainfall) to SSO IV (receiving 900 mm rainfall). Fertility variation, estimated as sibling coefficient, showed negative correlation with fecundity (ranging from 3000 fruits per tree in SSO I to 13 in SSO IV) and annual rainfall. There was no significant variation in fecundity and sibling coefficient (ψ) between two consecutive years.

Low fecundity was recorded in SSO 4 located at the arid site for two consecutive years (13 and 187 fruits per tree). The relative status number (Nr) size was ranging from 65 to 6 % decreasing from SSO I - IV. Tree growth and fruit production decreased with annual rainfall and hence a significant positive correlation ($R^2=0.45$) was observed between tree growth (DBH) and fecundity. SSO IV located at an arid site had high sibling coefficient values (16 & 8) and is not an ideal site to establish seed orchards of this species.

Key words: *Acacia auriculiformis*, seedling seed orchard, effective population, sibling coefficient.



A Program for Rapid Evaluation of Fertility Variation and Gene Diversity in Seed Stands and Seed Orchards

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Abstract: A program was developed to help seed orchard managers and tree breeders to evaluate the quality of seed stands and orchards based on tree fertility and adopt measures to improve gene diversity of seed crop. Individual tree fecundity values are provided as input along with the actual location of the tree in the orchard. The program computes sibling coefficient as a measure of fertility variation in the orchard. Along with fertility, variability in tree growth and form traits within the stand help us to decide the strategies for thinning the orchard. Growth traits like tree height and stem diameter, form traits like clear bole height and stem straightness and the reproductive output of each tree is assessed and entered in the spreadsheet of the software. The software calculates an index value for each tree based on the block adjusted value of each trait and a weightage factor (base on the correlation of the trait with stem volume and variability of the trait in each stand). The program will sort the trees according to index values based on which the number of trees to be retained in the stand can be decided. The position of the selected tree in the stand is also indicated as graphically by the program. Reproductive output of the selected trees can also be compared with the rest of the trees in the stand. The software enables rapid computing of orchard parameters which can be stored and retrieved easily. Information on orchard dynamics helps the orchard manager to make seed collection each year.

Key words: Thinning, seed stand, seed orchard, index selection, orchard dynamics.



Recurrent Selection and Genetic Thinning of Seed Orchards Through Estimation of GCA and Breeding Values from a F₂ Progeny Trial in *Eucalyptus tereticornis* Smith.

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Introduction: Precise estimation of fidelity of gains in progeny trials is the central dogma of modern breeding theory to improve the yield in terms of wood and its quality. Progeny trials are mainly used to evaluate gains achieved in the parental selection and also to direct the variation towards a particular outcome rather than random. Though randomness is main principle of modern evolutionary theory, if phenotypic adaptations are acquired as an average response to environmental perturbations and such variations live for a longer time, they may correlate with random genetic variations due to laws of probability (Simpson, 1953). If those traits were already exists in the population but not expressed in normal environments, they can quickly express under such circumstances and transforms into genetic trait and spreads in the population. However, due to trade-off between these two, variations made the individual to fit into its habitat (Hinton and Nowland, 1987, Sumita and Sindhu, 2011).

Seed orchards are considered as a gene package with improved seeds at the end of a generation of breeding. Through assessing the genetic value of a trait, inbreeding in the populations can be managed through thinning of inferior trees. Seed orchards are mainly designed to allow panmixis to its maximum. When selection in half-sibs is attempted in a progeny trial, the General Combining Ability (GCA) and breeding values (Best linear unbiased predictor) must be assessed because, GCA reflects interactions between alleles at gene loci and represents an average performance of the progeny of an individual when it is mated to a number of individuals in the field (Zobel and Talbert, 1984). GCA and breeding values were used for precise recurrent selection to increase the genetic gains.

Material and method: An *Eucalyptus tereticornis* F₂ half-sib progeny trial was established with 49 families selected from two seedling seed production areas and one seedling seed orchard located in three southern states of India. The orchards were already thinned and only best performing families were retained. The progenies were evaluated for their performance for fast growth and also for inclusion into F₃ breeding program. Based on the performance of progenies, GCA and breeding values of mother trees were estimated. The progeny trial was laid in RCBD with five replications. Each seed lot has seven plants assigned randomly within each replication and planted in contiguous rows with an espacement of 3 × 2 m. The mortality was stabilized at 4.77 % at age four years. The growth components such as total height, clear bole height and girth at collar were measured at the age of four years. Unbiased population variability was measured through coefficient of variation (CV) and intra-genotypic variation was analyzed by one-way ANOVA while GCA and breeding values were estimated as per Zobel and Talbert, (1984).

Results: Progenies exhibited good unbiased population variability for each growth component (Total height= 20.98, clear bole height = 21.1 and girth at collar = 19.63 %). Similarly, one-way ANOVA revealed highly significant intra-genotypic variation in all growth components suggesting that the progenies were responded positively to their environment and sufficient diversity within the population which can be exploited for further improvement (total height and clear bole height $p = < 0.003$; Girth at collar $p = < 0.001$). The genotypes exhibited wide range of GCA and breeding values.

Further, tree no. 17, 6, 8, 7, 22, 20, 43 and 44 has the highest breeding values for all three growth traits indicating linearity of the growth components. Tree no. 21, 19, 10 and 5 also possesses high breeding values for total height. Similarly, girth has linearity with total height (tree no.11) and clear bole height (tree no. 33) with higher breeding values. Tree no. 12, 15, and 14 had high breeding values for girth alone. Likewise, five trees possessing the lowest breeding values for each growth trait were also listed.

Discussion: Highly significant variation in the progenies suggested sufficient diversity within the population and such variation was common in species with exploitive and out-crossing breeding system growth strategy (Kozłowski and Keller, 1966). Eucalypts have exploitive growth strategy with indeterminate growth which results in lowest rate of bifurcation in the apical meristem. Growth in such species

is largely determined by current growing conditions. Disturbances in environment or micro-site effects in current growing season results in inducing higher variation in growth components (Hutchings and de Kroon, 1994) and may result in higher buffering actions during developmental stages which are the main reason for such higher intra-genotypic variability. Further, it was assumed that control by a set of pleiotropic genes which control individual component expression in such a way that well balanced genotypes are better able to buffer character development against environmental perturbations (Clarke, 1993).

It can be observed that all the best performers in terms of GCA for all growth traits were from Pudukotai SSPA indicating broad genetic base of the orchard. Like wise, the worst performers were from Karnataka SSO and Pondicherry SSPA suggesting narrow selection range or family co-ancestry in those parents and they can be culled to avoid further inbreeding effects (Soh, 1991). In addition to these, the parental orchards of these poor performers may be rejected for further improvement. With the increase in selection intensity, variations have a tendency to increase as a function of average response to environment and more precise selection may be made from these progenies for further improvement in growth. Best performers showed good values for all growth traits indicating correlated growth traits which facilitate simultaneous improvement of multiple traits and such strong age-age correlations for both height and mean annual increment were reported in *E. grandis* (Osorio *et.al.* 2003). However, due to non-linear nature of multiple alleles in later ages, the directions of change in gene frequencies are not constant and the alleles may change from positive to negative while phenotypic gains improve (Xu *et. al.* 2004). Hence, sufficient number of parents with high breeding values is to be selected. The breeding values in the present progeny trial favor selection of 10 parents having highest breeding values for each growth trait to synthesize the F₃ generation breeding population. In addition to these, a large number of progenies may also be selected with more precision through forward selection for maintaining diversity in the population.

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Correlation between Cone Production and Growth Characteristics in Clonal Seed Orchards of Scots pine

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Abstract: This study was carried out to find out the relationship between reproductive and vegetative growths in seed orchards. We surveyed cone production, height growth, crown diameter, basal diameter and diameter at breast height of 108 grafts from 36 clones in two seed orchards of Turkish Scots pine. There were positive and significant correlations between cone production and growth characters except height growth. Cone production was predicted based on the growth characters using regression analysis and fuzzy logic. While 72 data used for modeling, 36 data was used in predictions. Average of predicted cone production (77.7) per graft was similar to the averages from regression and fuzzy logic predictions, although there were large differences between empirical and predicted data of some grafts. The average cone predictions were 76.0 for height, 76.5 for crown diameter, 78.5 for basal diameter and 77.7 for diameter at breast height from regression analysis. They were 77.8, 78.1, 76.6 and 79.6 from fuzzy logic, respectively. Results of the study showed that predictor of cone production could change for the analyses, and both methods could be used for different purposes in seed orchards such as uncorrelated characters.

Key words: Fuzzy logic, artificial neural network, *Pinus sylvestris*, prediction, reproductive.



Randomized, Replicated, Staggered Clonal-Row Seed Orchard Design

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Abstract: Seed orchards' parents are often randomized to minimize selfing and promote cross-fertilization. When parents are unrelated, the randomization task is easily accomplished by the permuted neighbourhood design through the application of the **Computer Organised Orchard Layouts (COOL)** (Bell and Fletcher 1978). Advanced-generation orchards; however, pose a formidable design challenge as they often harbour related clones, a situation current designs could not handle. Although most conifers have several built-in selfing avoidance mechanisms including polyembryony, high genetic load, and within tree reproductive asynchrony, randomization still is the choice of seed orchardists in spite of its associated added management burden. Here, we present a new seed orchard design called **Randomized, Replicated, Staggered Clonal-Row (R²SCR)** that takes advantage of combining randomization with row arrangement. Rows are staggered so each clone is surrounded by four different clones and the neighboring clones vary across replications. Row length varies between a single and any even-number producing single-tree and clonal-row, respectively. The program permits equal, linear, and custom deployment options. For any selected deployment option, the resulting layouts are compared using the "minimum distance" concept (Lstibůrek and El-Kassaby 2010), the least square difference between the desired and actual clone size, and the number of empty spots the algorithm could not fill. The R²SCR is an interactive computer program designed to allow users to control any parameters combination including, orchard size and grid configuration (shape), row length, number of clones, minimum distance between ramets, and clonal deployment option.

Key words: Seed orchard design, randomization, clonal-row vs. single-tree, minimum inbreeding, equal, linear and custom deployment.

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Seed Orchards Utilized in Interspecific Fir Hybridization

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Abstract: Interspecific hybridization is generally considered to be one of the most efficient methods in forest tree breeding. Interspecific hybrids of firs produced so far were recommended as a substitute for declining silver fir (*Abies alba* Mill.) in central Europe. Its decline is ascribed to many factors including the reduced genetic variability of the domestic populations during the last centuries. The results of many studies indicate that interspecific hybrids of exotic species of fir seem to be prospective candidates for replacement of withering silver fir in the region.

Current activities are connected to a traditional hybridization program within genus *Abies*, which has been recently extended by a cooperation with an American partner (North Carolina State University in Raleigh, NC). This experiment is in the long run based on bilateral international cooperation between Czech Republic and USA. Czech side has been supported by a grant of Czech Ministry of Education within a Program KONTAKT. In this particular case there exists a concrete collaboration between the Czech University of Life Sciences in Prague (responsible researcher Prof. Jaroslav Koblíha) and the NCSU Christmas Tree Genetics Program (responsible researcher Prof. John Frampton).

All of the Czech seed orchards were founded as biclonal – grafts originated from 2 interspecific hybrids of the first generation F_1 *Abies cilicica* x *Abies cephalonica*. These grafts have fructificated many times and Prof. Koblíha has been experimenting with their control pollination since the 1980's. That way F_2 material and new interspecific hybrids were obtained. Part of this material is cultivated within the breeding station Truba, Kostelec nad Černými lesy. Owing to good experiences with coning and fertility of this material and also outstanding growth and vitality characteristics that suggested great potential for hybridizations, it was decided for further utilization of this material. Secondary grafts were taken to

establish mentioned hybridization seed orchards. These seed orchards primarily produced F₂ hybrids. European silver fir have been utilized as a rootstock in all of the cases.

Hybridization seed orchards coning before the year 2006 had been utilized mainly for production of F₂ hybrids.

Fraser fir (*Abies fraseri* (Pursh) Poir.) Christmas tree plantations in North Carolina are infested by root rot caused by *Phytophthora cinnamomi*. This disease kills almost 100% of Fraser fir material and leaves the soil indefinitely infested, thus unsuitable for replanting. In the experiment, control crossings of Mediterranean fir hybrids *Abies cilicica* (Toros fir) x *Abies cephalonica* (Grecian fir) with *Abies fraseri* were performed to obtain possibly resistant hybrid material of desired Christmas tree parameters. Pollen of various clones of Fraser fir and two clones of the Mediterranean hybrid (CZ1 and CZ2) was exchanged between CULS and NCSU. Control pollinations were performed annually in April/May 2007-2011 in the hybrid seed orchards as well as at Rattlesnake Knob clone bank in North Carolina. Annual cone collections were managed during September. After cones completely disintegrated the dry seeds were processed within each facility.

For the Czech material, basic parameters of cones and seeds were assessed for each seed lot. Crosses by CULS produced 176,300 seeds in the study period. Samples of the individual seed lots were X-rayed during October and November for the final step of full seed assessment. Generally obtaining 5% viable seeds in the sample can be rated as success. The average filled seed for each pollination year ranged from 1% to 3.6%. The best hybrid combination so far was CZ1 x NC73, yielding a predicted 16% viable seeds and 526 germinants (8.6%) in 2008. In comparison, the open pollinated (OP) crossing of CZ1 and CZ2 clones within the seed orchards had a 29% germination rate. The most successful tri-hybrid of 2010 was CZ2 x NC81 with a predicted 7% viable seeds, however germination of all the crosses was very low – 0 to 10 seedlings.

The hybrid material has been grown at both the CULS Kostelec Forest Breeding Station and in the greenhouses at NCSU. A subsample of seedlings grown from the 2008 control pollinations that were germinated in 2009 was measured for various traits in July of 2011. Open pollinated seedlings of the parent species *A. cilicica*, *A. cephalonica* and *A. fraseri*, were included in the measurements as well as OP F₂ seedlings. Measured traits were growth by year [summed for total height], needle length, number of lateral and whorl branches for 2009 and 2010, and the number of lateral and whorl buds for 2011. As expected the OP F₂ had a greater average height than either parent species.

Similar morphometric study will be executed during April 2012 within Czech facilities on a subsample of seedlings grown from the 2007 control pollinations that were germinated in 2008. F2 seedlings of the same age will be a standard material.

Growth traits of the tri-hybrid crosses when Fraser fir was the female parent followed a general pattern for all traits. The seedlings crossed with the Czech hybrids were intermediate between Fraser fir and the OP F2 in height, needle length, branch and bud numbers. When Fraser fir was the male parent of the tri-hybrid, the differences were more variable. All growth traits for tri-hybrids with Fraser fir as the male were lower compared to tri-hybrids with Fraser fir as the female. Tri-hybrid seedlings with Fraser fir as the male were taller than Toros or Grecian firs, but similar to the OP F2. Overall comparison of the species and hybrids showed Fraser fir as the fastest growing, fullest seedlings with the tri-hybrids exhibiting intermediate growth characteristics usually exceeding the Mediterranean and OP F2 seedlings. The most dramatic differences were seen in the lateral branch and bud numbers while needle lengths showed the least dramatic differences.

Breeding for resistance to *Phytophthora* root rot is a primary objective of the NCSU Christmas Tree Genetics Program. Parent species and samples of the tri-hybrids were included along with momi fir (*A. firma*) in a *Phytophthora* inoculation trial in 2010. Mortality of seedlings from crosses of the Czech hybrids with Fraser fir as the male parent ranged from 50 to 81 percent. Fraser fir mortality was 100%, while the mortality rates of Grecian, Toros and the OP F2 were more moderate. Momi fir has proved to be the most resistant in inoculation trials and had a 1% mortality rate in the 2010 trial. Resistance appears to be dominant perhaps under simple genetic control of 1 or a few loci. More testing of larger numbers of seedlings and the reciprocal crosses needs to be undertaken.

CULS and NCSU plan to continue the exchange of pollen and hybrid seeds. Single nucleotide polymorphic (SNP) DNA markers will be used to verify the tri-hybrids. Field plantings and subsequent measurement of growth and survival are planned for both hybrid material and the parent species. Vegetative propagation by somatic embryogenesis and rooted cuttings should provide a sufficient amount of material for *Phytophthora* screening and future field trials. NCSU is also undertaking the hybridization of Fraser with momi fir in cooperation with a Christmas tree producer and nurseryman. The investigators extend many thanks to the staffs of both universities and cooperating government entities.

Key words: seed orchards, fir, *Abies fraseri*, *Abies cilicica*, *Phytophthora cinnamomi*, interspecific hybridization.



Fertility Variation and Molecular Genetic Diversity in *Acacia leucophloea* Populations: Evidences for Outcrossing Behaviour

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Abstract: Tropical *Acacia* species have been regarded as pioneer species as they can adapt to a wide range of sites and soils. A large number of *Acacia* species have been recommended for forest plantations and rehabilitation of marginal and degraded ecosystems (Wickneswari *et al.*, 2005). The Indian *Acacia* encompasses a wide variety of species of which *Acacia leucophloea* (Roxb.) Willd. maintains a larger domain. It is well established that genetic diversity is the basis for the ability of forest trees to adapt to the changes that occur in their environment. Fertility and fecundity variations of this tropical arid zone species were estimated by assessing flowering abundance and fruit set pattern over a period of two consecutive years in four natural stands *viz.*, TNL 1, TNL 2, RKP and PDM of southern India. With the advent of DNA fingerprinting technique, molecular markers have been widely used to assess the genetic variation in tree species (Newton *et al.*, 1999; Kremer *et al.*, 2005). In this study, RAPD markers were employed to characterize and detect polymorphisms in five selected provenances of *A. leucophloea* namely Dharapuram, Thirumangalam, Pudukottai, Sendurai and Dharmapuri located in southern part of India. Genetic diversity was evaluated based on these two complimentary approaches and the results have been compared on the context of genotype and phenotype.

Among the four natural stands, three stands *viz.*, TNL 1, TNL 2 and PDM were situated in dry upland regions while location RKP is a waterlogged site. Natural fruit set was < 1%. Fruit production was low in RKP than other 3 stands. No year-to-year variation was observed on fruit set percent in all the stands. Correlation between growth (GBH) and flower production per tree in all the stands showed a

weak-positive correlation during both the years, indicating that tree diameter is not a constraint for flower production in the species. Similarly a correlation performed to check that if larger inflorescences by size produce more number of fruits, also failed. Invariably in all the four stands, ant-tree association could be observed in most of the individuals. Fertility variation calculated as sibling coefficient (ψ) was interestingly low in the stands except the location RKP which had ψ value > 4 . Male fertility variation ranged from 1.28-1.64 and do not vary much between the stands. The effective population size (N_s) was also low in RKP when compared with other sites predicting a high genetic drift. Similarly a high group co-ancestry (Θ) and low predicted gene diversity (GD) was also observed in this location.

Molecular diversity of four provenances was estimated using RAPD markers which exhibited 29.38% polymorphism. The genetic similarity coefficient values indicated that the provenances Thirumangalam and Dharmapuri were closely placed (1.00) while Pudukottai & Sendurai were distantly placed (0.545). However, all the provenances showed a 50% similarity between them. The cluster analysis separated provenances of *A. leucophloea* into 2 major clusters *i.e.*, Dharamapuram, Thirumangalam, Pudukottai and Dharmapuri into one cluster and separated Sendurai into another cluster. The present study has also generated a total of 36 Provenance Specific Markers (PSMs) of various molecular weights.

Relatively all the stands had a low fertility variation except the stand RKP. Even in this location, the combined fertility (male and female) was less than 2.5 which stood well below the sibling coefficient value ($\psi = 3$) as predicted for a better performing stand by Kang *et al.* (2003). This implies that the species harbours considerable amount of diversity. Also, the RAPD markers have demonstrated almost 30% of polymorphism within species. Hence it can be considered that the species is an outcrosser as reported in other Indian Acacias (Mandal and Ennos, 1995; Tandon and Shivanna, 2001; Solomon Raju *et al.*, 2006).

Key words: *Acacia leucophloea*, fertility variation, RAPD markers, outcrossing.



Seed Germination and Seedling Growth Attributes of *Terminalia chebula* and *Terminalia belerica* Under Different Pre-sowing Treatments

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Abstract: *Terminalia chebula* (Gaertn.) Retz. and *Terminalia belerica* Roxb are two important medicinal plant species used for various purposes in the Indian sub continent. The fruits of the species are widely used in combination with *Emblica officinalis* in *Triphala* in Ayurvedic medicine. However, due to tremendous population pressure, indiscriminate destruction of forest lands with inadequate quality seeds of the species and low germination capacity, the existence of these two vital tree species are at risk. Therefore, the present study was designed to accelerated the seed germination capacity and seedling growth of *T.chebula* and *T. belerica* with different pre-sowing treatments. Around half of the freshly collected ripe fruits of *T. chebula* and *T. belerica* were depulped by rotting fleshy pulp in water. The intact fruits or depulped seeds were dried and kept in airtight container until treatments were applied. The effects of depulping and soaking period on seed germination and seedling growth attributes were explored by soaking the dry intact fruits or depulped seeds in cold water for 0, 24, 48 and 72 hours and sown in the polybags filled with soil and decomposed cow dung at a ratio of 3:1. The study reveals that the fastest seed germination of both species was in depulped seeds soaked in cold water for 72 hours (DT3) and delayed germination was observed in intact fruits without treatment (IT0) for *T. chebula* and in intact fruits soaked in water for 48 hours (IT2) for *T. belerica*. Germination percentage varied from 36.67 to 73.32 in *T. chebula* and from 36.67 to 93 in *T. belerica* seeds. The highest germination percentage was observed in depulped seeds soaked in cold water for 48 hours (DT2) for both the species *T. chebula* (73.32) and *T. belerica* (93) followed by DT3 (70) for *T. chebula* and DT1 (85.6 percent in depulped seeds soaked in water for 24 hours) for *T. belerica*, which were significantly higher than

the other treatments including the controls (36.67 for both the species). Growth parameters considering shoot length, root length, total height, leaf number, leaf area, collar diameter and vigor index were highest in the *T. chebula* seedlings grown from the depulped seeds soaked in cold water for 48 hours and lowest was in the seedlings from intact fruit without treatment. Although, growth parameters like shoot length, root length, total height, leaf number, leaf area, collar diameter of *T. belerica* were highest in the seedlings developed from the depulped seeds soaked in cold water for 72 hours, the vigor index was highest in seedlings from depulped seeds soaked in cold water for 48 hours and lowest was in seedlings from intact fruit without treatment. The total dry mass per seedling was also highest in seedlings developed from the depulped seeds soaked in water for 48 hours for *T. chebula* and 72 hours for *T. belerica* and the lowest was in seedlings grown from intact fruits without treatment for *T. chebula* and 24 hours soaking in water for *T. belerica*. Therefore, considering the imbibition period, germination percentage, growth performance including vigor index and total biomass produced per seedling, pre-sowing treatment depulping the fruits and soaking in cold water for 48 hours may be recommended for the species *T. chebula* and *T. belerica* to obtain maximum germination performance.

Key words: Depulping, germination percentage, pre-sowing treatment, seedling growth.



Clonal Propagation of *Adhatoda vasica*, *Rauwolfia serpentina*, *Vitex negundo* through Stem Cutting in Low Cost non-mist Propagator

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Abstract: *Adhatoda vasica* (Basak), *Rauwolfia serpentina* (Sarpagandha) and *Vitex negundo* (Nishindha) are three important medicinal plant species used for many purposes in the Indian Sub-continent. The species usually grow naturally in the forest but due to tremendous population pressure, rural poverty, unsustainable utilization of forest resources and indiscriminate deforestation make these plant species vulnerable for their existence. Domestication outside of the forest through clonal propagation is one of the best options for the multiplication and restoration of the species. The present study describes the potentials of these three species for clonal propagation by stem cutting in low cost non-mist propagator for the domestication and mass production. The study reveals that all the three species were able to produce root in the stem cuttings even without rooting hormone. However, rooting ability was significantly enhanced in the cuttings of *A. vasica* and *R. serpentina* treated with rooting hormone IBA. Considering the rooting percentage, number of root produced per cutting, length of the longest root, stem cuttings without any exogenous rooting hormone is recommended for *V. negundo*, with 0.1% IBA solution for *A. vasica* and 0.4% IBA treatment for *R. serpentina* for mass clonal propagation. The survival percentage, initial growth performance of the cuttings of the species rooted under various treatments was also assessed in this study under nursery condition.

Key words: *Adhatoda vasica*, clonal propagation, *Rauwolfia serpentina*, rooting ability, stem cutting, *Vitex negundo*.



The Situation and Development of Seed Orchard in China

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Abstract: The history of tree improvement in China is much younger than many other countries with better forest management. There were a few experimental works by individuals in China in the 30s of 20th century. Ye Peizhong began hybridization within Chinese-fir (*Cunninghamia lanceolata*) in 1938, The first hybridization between poplar species was subsequently carried out in 1946. The first provenance trial had been set up for *Pinus massoniana* by Xinsui Yu in year 1957. The first conference in tree genetic improvement of China was hold by Forestry Society of China (FSC) in 1964 and some 109 papers were collected and published in 1966. The work of plus tree selection was begun in 1964. Thirty more plus trees in Chinese-fir were selected by Ye Peizhong, and then the first clonal seed orchard in Chinese-fir was settled in Fujian province. The first seed orchard for introduced pine species was set up by Zhisong Zhu in 1966 for loblolly pine. The National Cooperation Meeting of Tree Improvement was held in October 1972, Fujian province. The aim of the divisions of tree improvement was to develop strains of trees showing increased vigour, improved stem form, better adaptation to adverse conditions, increased resistance to pests and diseases and improved timber quality. The national wide tree improvement was begun. There were 40,000-50,000 plus trees in 40 more species had been selected and conserved in clone-banks and clonal seed orchard in 1970s and 1980s. Untested seed orchards had been setup and the area of total seed orchards reached 16,000ha in 27 provinces. All seed orchards are invested by the State Department of Forestry. Annual seed production in seed orchards is about 300,000-400,000 kg and seedlings of 20% new plantation, especial for coniferous species, come from seed orchard in 1990s. The genetic gains of timber volume in offspring of seed orchard are over 10% in general. Some should be above 15%.

However, the development of seed orchard was declined for government investment focused on a few so called 'fast-growth species' in 1990s, the management for other seed orchards was in very poor situation for less funding. The second spring for seed orchard in China may begin in year 2000s for "The Seed Law of the People's Republic of China" was published in year 2000, and that had made decisions for all seeds deployed in new plantation must be certificated of genetic improved varieties. After the law made, the first 131 state key improved forest germplasm production bases (seed orchard) of 48 species were selected and should be supported in financial by government in 2009. Advanced seed orchard program were launched in this species. Some species, as *Cunninghamia lanceolata*, had developed the third generation seed orchard.

The researches of tree improvement supported by national funding started in 1980s. The institutes of research in forest genetics and tree breeding are about 30 more as: the Chinese Academy of Forestry, Beijing Forestry University, Nanjing Forestry University, Northeast Forestry University, and provincial forestry research stations. Studies in tree breeding are focused on genetic improvement of major afforestation species of both coniferous and broad-leave tree species in China. The traditional breeding methods are coupled with modern biotechnology, physiological and ecological means to create modern breeding technologies in order to shorten breeding cycles and to improve the efficiency of breeding.

Key words: History, Tree improvement, Seed orchard, China.



Parental Reproductive Investment and Success in Conifer Seed Orchards

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Abstract: Reproductive success is the relative gametic contribution of a given parent to the next generation's gene pool. There are a variety of methods for predicting or estimating reproductive success in seed orchards. The simplest and least expensive method is based on the assumption that parental reproductive success is a function of parental representation, where the production of male and female gametes (fecundity) reflects the number of replicates (ramets) of a given parent (e.g. Lindgren and Matheson 1986) or their total crown volume (Burczyk et al. 1996). Other commonly used methods approximate reproductive success from reproductive investment and assume that there is a high correlation between fecundity and actual success (Gregorius 1989). These methods include visual scoring of generative buds or pollen- and seed strobili, measuring seed-cone volume or mass, counting the number of seeds per cone and estimating the proportion of filled seeds per parent, or classifying individual ramets into groups of similar fecundities. Sometimes, mainly for economic reasons, male fecundity is not assessed and is assumed to be equal to that of female. According to a number of published studies, substantial variation in both male and female reproductive investment has been observed among seed orchards' parents of many forest tree species. Using molecular genetic markers, a significant relationship was found between parental male-cone production and proportion of seed sired in white spruce (Schoen and Stewart 1986) and Douglas-fir (Burczyk and Prat 1997). In Nordmann fir (Hansen and Nielsen 2010), Japanese black pine (Goto et al. 2005), and sugi (Moriguchi et al. 2007), male fecundity explained 76, 43, and 15% of the variance in reproductive success, respectively. In all of these studies, however, almost an equal number of seed was used to represent each female parent; thus,

the generated information was only representative of the male reproductive success.

The objective of this study was to evaluate the accuracy of several methods for predicting female (F) and male (M) reproductive success. These methods are gender specific and include: a) parental representation (F0), b) visual seed-cone count on a ramet bases for each parent (F1), c) measuring seed-cones' volume and mass following harvest (F2 and F3, respectively), and d) seed-cone count calculated from the mass of a standard volume (F4) or e) from standard volume (F5) for female parents and a) parental representation (M0), b) parental representation followed by crown volume adjustment (M1), and c) visual assessment of pollen-cone production on 50% (M2) and 100% of ramets (M3) for male parents (see Woods 2005 for details). We also quantified the impact of each method on the genetic diversity expressed by effective status number (N_e ; Lindgren et al. 1996) and genetic worth estimates and determined the most appropriate methods for conifer seed orchard management.

We performed a linear regression of the reproductive investment data with data obtained from microsatellite-DNA-based pedigree reconstruction using seed crops – either clonally harvested or bulked over the orchards' populations – from four different species (lodgepole pine (*Pinus contorta* Dougl. ex. Loud. var. *latifolia* Engelm.), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), western larch (*Larix occidentalis* Nutt.), and western redcedar (*Thuja plicata* Donn. ex D. Don)). The DNA-based estimates were considered as a benchmark for evaluation, as they represented the actual parental reproductive success. In order to obtain unbiased estimates of the female component, we used random samples of bulk seed with unknown female and male parentage and independently analyzed embryos and their corresponding megagametophytes in Douglas-fir (El-Kassaby et al. 2010) and utilized paternally inherited chloroplast markers in lodgepole pine (Funda et al., in preparation).

Of the various tested methods, parental representation (M0 and F0) provided the weakest prediction of both male and female reproductive success, explaining 19 and 20% of the variance in reproductive success for the female component and 42, 31, 27, and 8% of the variance in reproductive success for the male component of Douglas-fir, lodgepole pine, western larch, and western redcedar, respectively. On the contrary, the inclusion of parental fecundities substantially improved the accuracy of predictions. For the female component, the explained variation increased to 89 and 66% for Douglas-fir and lodgepole pine, respectively, when seed-cone volume was used (F2) and similar improvement was also obtained by

seed-cone mass (F3). These findings indicate that as long as the number of viable seeds produced within a seed cone is proportionate to its size and/or mass, both methods F2 and F3 may be considered as reasonably accurate indicators of female reproductive success. Methods F4 and F5 seem to be unsuitable from a practical perspective, because they eliminated some parents due to the imposed seed-cone volume required for its use. For the male component, which is generally more difficult to predict, the explained variation increased as well and reached 55, 58, 73, and 61% in Douglas-fir, lodgepole pine, western larch, and western redcedar, respectively, when fecundity scores (M3) were utilized. The improvement was particularly appreciable for western larch and western redcedar, indicating that male fecundity variation is a very important factor to be considered. Furthermore, high correlations between fecundity scores from M3 and M2 in all studied species indicated that scoring of just 50% of ramets may suffice to provide reasonably accurate estimates of the total pollen production in seed orchards, because it does not compromise the estimates' accuracy despite the reduced effort. It is also worth noting that a simple crown volume adjustment (M1) substantially improved the prediction as compared with M0 (50 versus 27% variation explained in western larch), so this method could be utilized as an alternative in situations where no male fecundity data is available.

Genetic worth estimates calculated from the survey methods did not differ significantly from those obtained from pedigree reconstruction due to a narrow range of the parental breeding values. On the other hand, the effective number of parents (both male and female) was overestimated in most instances. The greatest overestimation was detected in the female component of Douglas-fir (38.5 and 13.8 for F0 and F2, respectively, vs. 6.5 using pedigree reconstruction). The very low value of 6.5, surprising for an orchard with a census of 49, was due to an immense imbalance in reproductive success, as the most successful single female had produced more than one third of the total seed crop. For all of the four studied species, N_e estimates were most inflated by parental representation, while accounting for fertility variation provided estimates much closer to the actual values.

In conclusion, methods F2 (seed-cone volume) and M2 (partial pollen survey) provided reliable proxies to the actual female and male components of parental reproductive success, respectively, as well as to genetic gain and diversity of the studied seed crops and thus appear to be most appropriate for utilization by seed orchard managers.

Key words: Reproductive investment, reproductive success, pedigree reconstruction, microsatellite DNA, conifer, seed orchard.

Acknowledgements

Funds from the Forest Genetics Council of British Columbia to YAE and the Four Year Fellowship by the University of British Columbia to TF are greatly appreciated. We thank to Annette Van Niejenhuis, Cathy Cook, Hilary Graham, and Chris Walsh for parental fecundity data and seed collection and to Cherdasak Liewlaksaneeyanawin, Charles Chen, Ben Lai, Irena Fundova and Chakrit Na Takuathung for assistance with lab work and pedigree reconstruction analyses.

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Breeding and Seed Orchards of Turkish Red Pine in Turkey

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Abstract: Turkish red pine (*Pinus brutia* Ten.) is an economically the most important forest tree species in Turkey. It occupies 25 % of total forest area of the country. Since 1950, Turkish red pine has been used in 40 % of plantations in Turkey. It is also one of the fast growing tree species of Turkey and constitutes about half of logging production done by General Directorate of Forestry which is the biggest producer in Turkey.

Turkish red pine tree breeding in Turkey have been started with other main conifer species in 1960's. Seed transfer zones have been determined and seed stands selected in seed transfer zones within this breeding period. The first commercial seed orchard established by Turkish red pine in 1976. Studies based Seed Sources Planning were carried out until 1994. During the period Turkish red pine seed orchards occupying 320 ha area have been established.

The first detailed Breeding Program has been put into practice in 1994. *Pinus brutia*, *Pinus nigra*, *Pinus sylvestris*, *Cedrus libani* and *Fagus orientalis* were determined as target species in the program. Turkish breeders became familiar to progeny trial and gene conservation concepts by the initiation of this program. Great attention has been given to Turkish red pine because of its economical importance and biological superiority.

The first Turkish red pine progeny trial was established in 1998 in the concept of Breeding Program. Area of progeny trials has been reached to 80 ha by the end of 2010. Totally 1595 plus trees has been moved to trials. Randomized block design has been used in trials. At least two progeny trials have been established in each breeding zone and 7-15 blocks have been used in each trial. Therefore, each family was represented by 28-30 individuals in each trial.

According to results of progeny trials individual heritabilities were estimated between 0.15-0.42 at 4th year, between 0.16-0.27 at 8th year trials for height and between 0.12-0.22 at 8th year trials for diameter. Family heritabilities were

estimates as 0.42-0.76, 0.60-0.72 and 0.54-0.64, respectively. Estimates of results of trials demonstrated that genetic correlation between height and diameter is close to 1. Therefore, it is possible to make indirect selection by each character. Genetic correlation estimates between 4th and 8th year results were ranged from 0.84 to 0.95. According to this result, it is possible to make selection for 8th year at age 4. In addition, 12th year's measurements have been taken for the first progeny trials. Estimates for age-age correlations will also be evaluated for 12th years.

Seed transfer regions have been extended to breeding zones in the breeding program. Genotype-environment interactions estimated by progeny trials in new breeding zones were not significant to effect applications and therefore breeding zones were confirmed by these results.

Genetic gains were estimated by comparing to seed stands. Genetic gain estimates were done for seed orchard establishment in case of use of 30 plus trees with the best breeding values. Genetic gains were also estimated for existing seed orchards in the case of roguing. Genetic gain attained by unit period is important in breeding. Therefore, seed orchards were established by using families having the best breeding values according to the first results of each breeding zone's trials. More than 30 families exist in these seed orchards. So, it will be possible to carry out roguing in accordance with future estimates. In addition, seed production will be done in seed orchards after getting results of progeny trials.

Future plans in breeding activities constitutes establishment of 450 ha first generation genotypic seed orchards which will be substituted for existing phenotypic seeds orchards and later on establishment of second generation seed orchards.

Key words: Breeding, Seed orchard, Progeny test, Heritability, Genetic correlation, Breeding zone.



Management, Genetic Gain and Genetic Diversity in Clonal Seed Orchards in Croatia

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Abstract: In accordance with the delineation of Regions of Provenances of forests in Croatia into ecogeographic seed regions and zones, the following productive clonal seed orchards have been established: three orchards of pedunculate oak (*Quercus robur* L.), two of narrow-leaved ash (*Fraxinus angustifolia* Vahl), one of sessile oak (*Quercus petraea* (Matt.) Liebl.), one of wild cherry (*Prunus avium* L.) and one orchard of black pine (*Pinus nigra* J. F. Arnold). Phenotypical selection and heterovegetative propagation of plus trees, as well as the establishment of clonal seed orchards were launched with the goal of controlling more regular yield periodicity and obtaining forest seed of good genetic quality in the categories Qualified and Tested. The orchards are regularly subjected to pomotechnical treatments, protection and other measures of maintenance. Establishment and evaluation of progeny trials are provided for all clonal seed orchards.

Different pomotechnical treatments (training systems) were applied to each tree species. For pedunculate and sessile oak oval spindle training system, for narrow-leaved ash spindle pyramid and for wild cherry spindle bush training system. Training systems should provide a strong framework and good light penetration in the canopy. The forming pruning is used to bring the grafts into the required training shape whereas pruning for higher yield maintains the adequate balance between the growth and the seed production. Tree pruning and training started right after the planting with a goal to gain desired canopy shape with well-deployed scaffold branches in the next 7 or 8 years. The main function of pruning is the removal of competing shoots to enforce the growth of remaining desired ones. Pruning intensity, i.e. the relation between vegetative and generative buds in the canopy determines the tree's condition, density and yield. The balance between

vegetative and generative buds can only be achieved by appropriate underground and aboveground tree parts pruning.

The evaluation of genetic values of selected clones of pedunculate oak (*Quercus robur* L.) from seed orchards in progeny tests were also started for the purpose of obtaining increased genetic gain. Three open pollinated progeny trials have been established, representing three seed region at the age of 2 + 18 years (TP1) and at 2 + 7 years (TP2, TP3). The realised gain from the tests and expected genetic gains for height were calculated by three methods which included: realised gain (R) calculated as the difference between means of selected plus tree progenies and means of unselected control plants; expected genetic gain by individual within provenance mass selection of first generation plus trees at the same ages as those represented in studied trial; expected genetic gain by backward selection among first generation plus trees. In TP1 i TP3 family effect for height was statistically significant for the entire period, showing decreasing trend in TP1 and rising in TP3. Family effect was not significant in TP2, pointing to narrowed individual variability of plus trees in that seed region. High values of environmental variance components and family \times block interactions suggest strong environmental influences in that particular trial (caused by flushing phenology), which could have led to underestimation of additive variance. High values of additive coefficients of variation in TP1 and TP3 suggest wide genetic variability of selected plus trees and good adaptability of their progeny to changing environmental conditions. In all trials the estimated expected genetic gains by backward selection among first generation plus trees after open-pollinated progeny testing showed the highest values. Realised gains were not very high during the entire period in TP1, showing the decreasing trend in recent years. In TP3 realised gain is improving, due to higher heritabilities and greater differences between selected and control families. Opposite to that, low heritabilities, non significant family variance component and small differences between selected and control families resulted in little, even negative realised gains in TP2.

Genotypes from the clonal seed orchard of wild cherry (*Prunus avium* L.) was taken for the investigation of genetic variability and consisted of 24 selected plus trees from the area of north-western Croatia. The clones were analyzed by 15 selected microsatellite markers (SSR), chosen by the ECPGR. A wealth of allelic variations was found in SSR loci, while a high degree of polymorphism confirmed the existence not only of extensive morphological but also a very significant genetic diversity. Based on pairwise proportion of shared allele distance (D_{PSAM}) the average genetic distance of 0.573 was calculated. The genetic distance matrix,

based on pairwise proportion of shared allele distance (D_{PSAM}), did not show a clear classification of wild cherry individuals with regard to their origin, i.e. region (Koprivnica, Bjelovar, Zagreb). The analysis of molecular variance (AMOVA) revealed a significantly higher percentage (95.88 %) of the total microsatellite diversity caused by the differences among the individuals within the regions, compared to that caused by the differences between the studied regions (4.12 %). The ϕ - statistics, amounting to 0.041, was highly significant ($P < 0.01$) and indicates the existence of specific regional structularity of genetic diversity.

Clonal seed orchards are also a nucleus of conservation of forest genetic resource using the *ex situ* statical method, since the relationship between the size of population and the percentage of preserved heterozygosity is thus reduced to minimal loss of total additive genetic variability. Climate changes and new habitat conditions will pose additional challenges to seed production and forest management; in turn, this will influence their economic and social benefits, as well as biological diversity of forest ecosystems.

Key words: *Quercus robur*, *Quercus petraea*, *Fraxinus angustifolia*, *Prunus avium*, *Pinus nigra*, pomotechnical treatments, progeny trials, genetic parameters, genetic diversity.



Possibilities to Use $GA_{4/7}$ for Flower Stimulation in Seed Orchards in Sweden and the Rest of EU

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Abstract: Conifers of the *Pinaceae* family were first successfully induced to flower by gibberellin application in the mid-1970s, when the use of the relatively non-polar gibberellins GA_4 and GA_7 started. From that time and onwards numerous of research experiments using $GA_{4/7}$ for flowering stimulation has been performed, and generally with encouraging results. But practical use of the developed methods has been prevented because there has been no registered product available for use in seed orchards.

Together with GLOBACHEM the Swedish seed orchard managers and Skogforsk have worked with the process of register Gibb Plus Forest as a $GA_{4/7}$ product for use in flowering stimulation of conifer seed orchards. The application was approved by KEMI (The Swedish chemicals agency) in December 2011 and the approval is valid to 2019.

The fact that Gibb Plus Forest is approved for use in seed orchards in Sweden also opens possibilities to use in other EU countries.

In 2010 Skogforsk started an experiment where the effect of GA applied as Gibb Plus Forest is compared with GA applied in other solutions. And during 2011 Skogforsk and the Swedish seed orchard managers started to test and develop appropriate application equipment and application technique for Gibb Plus Forest. Results from these tests will be presented.

Key words: Gibberellin, $GA_{4/7}$, Seed supply, seed orchard management, Gibb Plus Forest®



Improved Forest Regeneration Material in Sweden 2010-2050 – Supply and Needs, and Measures to Minimize Shortage and Maximize Genetic Gain

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Abstract: The use of improved plant breeding stock is an effective way to increase wood production and thereby long-term harvest potential in Swedish forests. Today, forest owners are very aware of, and are very willing to use, improved plant breeding stock, but greater utilization is limited by the supply of improved seeds. An analysis of seed supply shows that the primary shortage involves seeds of Norway spruce. Currently, only one of nine spruce orchard zones has significant surplus of improved seeds. In the most northerly seed orchard zones, and in Götaland and milder parts of Svealand, there is a major deficit. The situation will improve somewhat in the slightly longer term, but it is still far from satisfactory. Not until around 2030 will supply meet needs throughout the country. The supply balances are based on extending the utilization time of the oldest seed orchards with relatively low improvement gains, so future wood production will be lower than if the programme for new seed orchards had been more ambitious. In the short term, the supply of Norway spruce seeds can be significantly improved with relatively simple and well-known methods. Seed production can be increased at low marginal cost through more intensive orchard management, stimulation of flowering and control of seed-destroying insects. These measures are limited by obstacles such as access to approved substances and financial constraints. Approval of a substance to stimulate flowering is urgent, and in December 2011 Gibb Plus Forest were approved for use in conifer seed orchards. In order to meet

the shortage of improved Norway spruce seeds, and to attain higher wood production, vegetative propagation of highly improved seed batches is realistic. The plant production cost is high, but calculations show that it would be viable because of the increased production that can be attained.

For Scots pine the analysis shows that, at present, the only shortage of orchard seeds for plant production is in the two most northerly orchard zones, while other parts of the country completely cover needs or even have a surplus. Around 2020 the northernmost zones for Scots pine will completely meet needs. If production-enhancing measures are implemented in the seed orchards in these zones, needs could be met a few years earlier.

The analysis is based on production in existing seed orchards and in those that are being set up in the national plan for the third stage of seed orchards. The analysis shows clearly that there will be another acute shortage of improved Scots pine seeds from orchards at the start of the 2040s and of Norway spruce seeds in the mid-2040s. It is therefore important to set up new seed orchards already now. This should be done continually in line with advances in plant breeding.

Key words: Seed supply, seed orchard management, genetic gain, flower stimulation.



Effects of Top Pruning on Flowering and Growth of Scots pine (*Pinus sylvestris* L.) in Seed Orchard

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Abstract: The aim of the study was to examine the influence of 2 top pruning intensity variants on flowering and subsequent growth of Scots pine. The experiment was carried out in seedling seed orchard in Głogów Forest District, established in 1996 from generative progeny of plus trees from south-western Poland. The mean height of trees was almost 7 meters. In winter 2005/06, the first turn of cuttings has been done in seed orchard and every second tree in every second row was cut, what constituted about 25% of total number of trees. In March 2006, in 2 of 4 blocks of seed orchard, every second tree in every row without cuttings was top-pruned. The total number of 279 trees were treated. Two top pruning intensity variants were applied, each in 3 replications. In the less intensive variant 1 (V1), the 3 top whorls have been cut just above the fourth whorl, in the more intensive variant 2 (V2), additionally the last whorl on every branches from the fourth and fifth remaining whorls has been also cut. The fungicidal paste was used to protect the injuries against infections. Untreated trees growing in the same rows with top-pruned trees were taken as controls.

In the next year after treatments (May 2007) and after 3 years past top pruning (May 2009), the male and female flowering of examined trees was estimated. The quantity of pollen strobilus clusters was visually scored based on their arrangement in the crown and the seed cones were approximately counted, both being included to proper grade of flowering scale. Male flowering was estimated in 6-graduate scale: 0- no pollen strobilus clusters; 1- pollen strobilus clusters only on few branches (up to 20 units); 2- pollen strobilus clusters located thinly at the bottom of the crown (20-100 units); 3- pollen strobilus clusters located thinly at the bottom and in the middle of the crown or densely at the bottom of the crown (100-500 units); 4- pollen strobilus clusters located densely at the bottom and thinly in the

middle of the crown (500-1000 units); 5- pollen strobilus clusters located densely at the bottom and in the middle of the crown (over 1000 units). The female flowering was estimated in 10-graduate scale: 0- no seed cones; 1- from 1 to 2 seed cones; 2- 3-5 seed cones; 3- 6-10 seed cones; 4- 11-20 seed cones; 5- 21-50 seed cones; 6- 51-100 seed cones; 7- 101-150 seed cones; 8- 151-200 seed cones; 9- over 200 seed cones.

After four growing seasons, in October 2009, the DBH and the height of trees were measured, the increment of DBH and height was calculated and the number of leader stems was counted.

The statistical analyses for flowering and the quantity traits were done using the Kruskal-Wallis non-parametric test. The significance level for all tests was $P = 0.05$. In the next year after top pruning, flowering in the investigated seed orchard wasn't intensive, especially male. There was no statistically significant difference in mean grade of female flowering between top-pruned trees in both variants and control trees. Whereas, top-pruned trees had in average more pollen strobilus clusters than control trees, but significantly only in variant V2. After 3 years past top pruning, the intensity of flowering in seed orchard was similar to that observed in the first year after treatments. The mean grade of female flowering in the less intensive variant V1 was significantly lower than in control trees. Top-pruned trees in both variants had in average more pollen strobilus clusters than control trees, but differences were not statistically significant.

None of applied top pruning variants resulted in increasing the mortality of trees. Only one top-pruned tree died, but the same was also for one control tree. After one year past top pruning, the mean DBH of top-pruned and control trees was similar, but after 4 years past top pruning, the top-pruned trees in both variants had significantly lower mean value of DBH. Top pruning significantly decreased the mean DBH increment in 4 years after treatment. The mean height of control trees and trees destined for top pruning did not differ significantly before the treatments. In variant V1, the height of trees has been reduced by top pruning by mean 2.83 m, and in variant V2, by mean 3.14 m. After 4 years past top pruning, the top-pruned trees were still significantly lower than control trees as a result of treatment, but the height increment of top-pruned trees was significantly higher towards controls, and after 4 growing seasons top-pruned trees exceeded the height from before the treatment by over 1 meter.

Both variants of top pruning resulted in increasing the number of leader stems of top-pruned trees. The consequence of the treatments was that the most of branches from the remaining highest whorl bent upwards and start to grow as the

leader stems. This increased the percentage of trees having 2 or more leader stems.

Summarising, top pruning reduced the height of trees in seed orchard, but the height increment was increased as a result of the treatment and trees recovered their previous size already in a few growing seasons. So, top pruning could not be the single treatment and have to be repeated to effectively retard the height increment of trees in seed orchard. The disadvantage of top pruning was that the number of leader stems in top-pruned trees was doubled or even more. The increased number of stems need for their growth increased amount of nutrients, what may causing a decrease in amount of nutrients accessible for seed production. In consequence, seed production after top pruning may be reduced.

Key words: Scots pine, seed orchard, top pruning, flowering.



Seedling Seed Orchards in Poland

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Abstract: In Poland often seedling seed orchards are established as objects combining production of improved seeds with genetic testing. Presently there exist 114 seedling seed orchards on 735 ha for 14 forest tree species. Most use open pollination progenies from trees phenotypically selected in forests. All SSO are designed in spacing 5 x 5 m. No crown pruning has been applied. The first thinnings are planned after 10 – 15 years. SSO are used for seed production, but in practice they are utilized only partly. It depends mostly on seed demands. But also on a subjective feeling of managers that using these seeds causes reduction of genetic diversity in plantations. Scots pine constitutes 34% of the total SSO area. In total 8% of seeds origin in 2010 from seed orchards and from this about 40% is estimated as seeds from SSO.

In a Swedish small trial (Rosvall and Lindgren 2012) comparing Scots pine seedlings and grafts in seed orchard conditions at Umeå, the seedlings developed pollen and seeds late, but seemed able to reach similar levels as grafts two decades after planting. Thus the results indicate that good seed production may be obtained from SSO, but there is a delay in delivery of seeds compared to grafted seed orchards. Selfing is expected to be lower than in a clonal seed orchard as there is only one tree of each genotype. The influence of the sib mating is reduced by unrelated pollen contamination from outside the orchard. Inbreeding depression on seed production, seedling mortality in the nursery, natural regeneration in planted stands and compensatory growth from non-inbred trees will reduce the impact of inbreeding on forest production, but for optimizing reasons considering diversity it is simple to assume 1 % inbreeding = 1 % production loss, which is an overestimate which guarantees that inbreeding and diversity do not get too little

weight. The inbreeding depression will be small if the number of families in the SSO is large enough.

It is suggested that phenotypic selection in half-sib progeny tests or plantations - even with unknown pedigrees - can be used to manage seed collection areas or as a component in low-input breeding without a fast buildup of coancestry or inbreeding, provided the initial number of progenies of unrelated parents is sufficiently high (Fedorkov et al. 2005).

In this paper using routinely collected data before planning selection thinning on SSO (Kowalczyk 2008), selection indexes were calculated: phenotypic (mass selection) (PH), individual tree, individual selection (IS), family selection (FS), family plus within-family selection (FWFS) and combined selection (CS). Selections were done with several intensities from 1% to 30% on the basis of index values. Effective family number and status number was calculated as parameters describing genetic diversity. The average gain was 4% to 35% depending on the method. Obtained results demonstrated as expected that combined selection provided the biggest genetic gain, although at a large cost in diversity lost. Family selection is limiting the genetic diversity. Individual selection (mass) is assuring large genetic diversity. If the aim to keep the genetic diversity is a priority, individual selection is being recommended. Combined selection and family selection limits diversity and for a given status number mass-selection is better or as good. The gain cannot be very high from a SSO with open pollinated progeny. Mass selection is simplest and it is easiest to apply thinning and when also consider spacing and seed orchard feasibility of the selections, thus this may be a preferred method. The SSO may first be thinned and when better trees may be harvested to further improve gain with limited loss of diversity. This study supports using the FRM from SSO and indicate possible ways to tradeoff between gain and diversity in practice.

SSO could be a part in long term breeding, where the best trees are grafted and used in seed orchards and as mother for crosses, while pollen is harvested from later selections and used as fathers. When SSO combined with progeny tests is established, and the procedure can be repeated many times. SSO can also support sustainable low budget long-term breeding without crosses or grafts.

Key words: Seedling seed orchards, genetic testing, index selection, mass selection, pollen contamination.

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Seasonal Variation in Saponin Content in *Asparagus racemosus* Breeding Arboretum

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Abstract: *Asparagus racemosus* (Willd.) is the priority species for cultivation and conservation selected by National Medicinal Plant Board (NMPB), Ministry for Health & Family Welfare, Government of India. It is an important medicinal plant is used in the Ayurveda, Siddha and Unai, traditional medicine systems in India. The tuberous root of the plant contains saponin which is used for medicine. The roots are used mainly to promote the secretion of breast milk, improve lost body weight and considered as an aphrodisiac. The demand of the plant can not met under natural condition. The species is categorized as threatened due to over-exploitation.

Forest Research Institute, Dehradun, India has undertaken a project for collection of plant of germplasm from different agro-climatic conditions its genetic improvement. A field trial of 20 sources has been established at the campus of this Institute to find out the sources/genotypes having more root production and total saponin content for production of improved genotypes through breeding. In this paper the variation between different sources in regards to root production and saponin content has been shown. The study was also undertaken on seasonal variation on production of total saponin content.

Key words: *Asparagus racemosus*, threatened, Genetic improvement, ex- situ conservation, saponin.



Effect of Different Climatic Conditions on Phenological Assortative Mating in a Black Pine (*Pinus nigra* Arn.) Clonal Seed Orchard

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Abstract: *Pinus nigra* Arn. (black pine) is an important and prominent high elevation pine species that is used extensively for reforestation purposes in the Greece due to its high ecological value and wood production. The need for improved genetic material is covered by a network of black pine first generation clonal seed orchards. For this purpose sixty plus trees were selected from five geographic regions representing the distribution of black pine in Northern Greece that were then used to establish a clonal seed orchard. Each clone was represented by sixteen to nineteen ramets that were planted following a honeycomb experimental design, so that kinship could be avoided. Main objective of a seed orchard is the production of genetically improved seed assuming that the criteria for panmictic equilibrium are fulfilled. Change of climatic conditions may be proved a crucial factor resulting in discrepancies in gamete gene pool composition and disturbance of phenological assortative mating among clones of a seed orchard, and thus affecting significantly the quality and quantity of the seed crop. Also, assortative mating due to flowering phenology can strongly influence the response to selection by increasing the rate of response to directional selection. This study is focusing on the role of temporal phenological assortative mating in shaping the mating patterns among the seed orchard clones for the two contrasting in climatic conditions years and its effect on the seed crop produced. The effect of the xerothermic climatic conditions versus normal climatic conditions on the flowering of clones that resulted in phenological disassortative mating was revealed. Disturbance of assortative mating and unequal parental contribution need to be seriously considered when management actions are planned, as they can affect significantly the genetic diversity of the seed crop. The impact of changing climatic

conditions though may be detrimental for the quantity, quality and genetic diversity of the produced seed.

Key words: flowering, assortative mating, climate change, reproductive output, parental contribution.

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Tree Improvement: Crossroads between Conventional and Free-Pedigree Breeding

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Abstract: Traditional breeding is accomplished through the selection of elite genotypes after ranking parents and offspring's breeding values. This is commonly done using the intra-class correlation approach, thus the generation of structured-pedigree is an essential prerequisite for its accomplishment. Structured-pedigree is not only needed for quantitative genetics parameters estimation but also is of a substantial importance for the management of co-ancestry, thus inbreeding is strategically managed (Lindgren and Mullin, 1997, Hallander and Waldmann, 2009). As breeding programs advance, the build-up of co-ancestry is unavoidable, unless gain is intentionally sacrificed. With the exception of the Best Linear Unbiased Predictor (BLUP) approach, that is capable of accounting only for known genealogy, most quantitative genetics data analyses focus exclusively on the genealogy of the parental population. Classical relationship estimates (Henderson, 1976), while useful, have their limitations specifically in complicated multi-generational pedigree. For example, relationship methods do not differentiate among members of a half-sib or full-sib family and are given similar relationship estimate (i.e. adjustment for Mendelian sampling term). Pedigree reconstruction helps in avoiding expensive controlled mating and simplify testing while provides better genetic diversity control (El-Kassaby and Lstiburek, 2009) but the genetic covariances still are not adjusted for the Mendelian sampling term. The availability of DNA genotyping and/or genomic sequencing data provided a powerful tool allowing greater relationship differentiation even among members of a single full-sib family as their relationship can be traced back to many generations. Comparisons between the use of genomic and traditional relationship estimates indicated that the former is much more superior to the later and revealed hidden

and often undetected quantitative genetics differences (Frentiu et al., 2008). The use of DNA genotyping and/or genomic relationship estimates frees breeders from the arduous task of tracking pedigree, which in this case, is considered as a limitation rather than advantage. Relationship estimates provide better information for both quantitative genetics parameters estimation and inbreeding management. Traditional breeding can be substantially simplified through the use of DNA genotyping and/or genomic information thus offering independence from structured-pedigree without any sacrifice to the accuracy or precision of the generated genetic parameters. The use of relationship estimates is expected not only to simplify tree breeding but also to create new opportunities that could not be contemplated through traditional tree improvement approaches. In this paper, the quantitative genetic parameters and their precision were estimated for various progeny testing experiments under different pedigree information. The data were analysed and compared for a continuum of family structure progressing from open-pollinated to full-pedigree generated through pedigree reconstruction (El-Kassaby et al., 2011, Kruuk, 2004) and ending with completely pedigree-free model using molecular- or genomic-based realized relationship matrices (Frentiu et al., 2008).

Key words: Breeding without breeding, marker-based kinship estimates, pedigree free model, genetic variance.

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Norway Spruce Indoor Seed Orchard for Production of Base Material for Cutting Propagation

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Abstract: In bulk propagation of cuttings, twigs from young seedlings are used, since they give higher rooting and less plagiotropic growth than cuttings taken from older plants. Thus, bulk propagation requires a supply of seeds from the best available plus trees. One way to effectively produce the base material for cutting propagation projects are indoor seed orchards with potted trees. In Sweden 1997 - 2004 Skogforsk in cooperation with four forest companies establish and managed an indoor seed orchard in central Sweden aiming to produce seed for bulk cutting propagation in operational scale. In connection to the establishment of the orchard research and development activities where performed.

Although is called an indoor seed orchard the potted trees are actually grown outdoor for most of the time. The trees undergoes a 3-year cycle: year 1; Flower stimulation in the greenhouse: protocol includes root pruning, reduced watering, heat treatment and stem injection of GA_{4/7}, year 2; Flowering and pollination in the greenhouse. Trees are transferred into the greenhouse early in spring to ensure flowering and pollination is completed before flowering starts outdoors. In this way we avoid pollen contamination. After flowering the trees are transferred outdoors, where cones and seeds develop, and year 3; a recovery year where the trees are grown outdoors for the whole year.

Economic calculations based on the experiences from our project shows that it is possible to produce base material for operational bulk cutting propagation.

Key words: Indoor seed orchard, seed orchard management, flower stimulation, Gibberellin, GA_{4/7}, cutting propagation.



Heritability in *Pistacia atlantica*

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Abstract: In the genus of *Pistacia* which belonged to the family of *Anacardiaceae* there are 11 species which some of them used as ornamentals and some valued as fruit tree. *Pistacia vera* L., *Pistacia khinjuk* Stocks, *Pistacia atlantica* Desf. and *Pistacia mutica* species grown naturally in Iran (Ahmadi Afzadi *et al.* 2007). *Pistacia atlantica* Desf. (Wild pistachio), locally known as “Baneh” in Iran, is economically the most important species for rural in forest areas of the Zagros Mountains in Iran (Pourreza *et al.* 2008). The resin of wild pistachio, called Saqez, is used for a variety of industrial and traditional uses, including food and medicine (Pourreza *et al.* 2008). *P. atlantica* is also used as a rootstock for edible pistachio tree (*P. vera* L.), (Kafkas and Perl-Tereves 2001; Kafkas *et al.* 2002; Barazani *et al.* 2004). As well as adaptation of *Pistacia* trees to harsh desert conditions, their longevity also make them ideal candidates for reforestation in arid zones (Golan-Goldhirsh *et al.* 2004). In spite of its various uses, no efforts for the genetic improvement of this species have been made (Golan-Goldhirsh *et al.* 2004; Javanshah *et al.* 2006). The study of heritability and genetic advance is very useful in order to estimate the scope for improvement by selection (Safavi *et al.* 2011).

So, the aim of this study was to find: (i) heritability of *P. atlantica*, (ii) relationship between seed characteristics with growing characteristics of *P. atlantica* seedlings. For this reason seeds were collected from 60 randomly selected trees of wild Pistachio (*Pistacia atlantica* var *kurdica*) of natural populations in northwest of Iran. Then seeds were sown in plastic pots in forest nursery. Thirty seeds were sown from each of the mother trees in a randomized complete block design with three replications. Ten seeds from each mother tree were randomly allocated to per replication. Collar diameter (cm) and seedling height (cm), were recorded when the seedlings were two years old.

Based on the results, maximum value of Heritability is for plant height. High heritability indicate that the selection for plant height will be effective being less influenced by environmental effects. And also reflects superiority of selection for plant height.

Significant positive phenotypic correlation coefficients were estimated between collar diameter seed size and seed weight ($r = 0.42$). Heavier seeds had progenies with larger collar diameter. Also there was significant positive correlation between plant height and maximum diameter of seed. Phenotypic correlation coefficients are mainly used for indirect selection in plant breeding programs (Chaturvedi and Pandey 2004). Based on progeny test we found useful information for early growth stage selection. Estimated heritability values indicated possibilities for improvement by utilizing existing genetic variation. Finally, plant height can be used as selection index because of its high heritability. Knowledge of complex relationship between vegetative characters of wild Pistachio tree would be essential to effectively select superior mother plants for developing seed orchard of the species by which fast growing seedlings could be produced.

Key words: Collar diameter, Heritability, *Pistacia atlantica*, Selection Index, Seedling Height.

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A Plan for the Second for the Second-Generation Seed Orchards of European Larch in Romania

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Abstract: Although it is one of the most important coniferous species, European larch (*Larix decidua* Mill.) covers a restricted area in Romania, only 0.3% of the forest area, most being represented by the artificial stands.

The natural area of the European larch is fragmented, being concentrated into 5 genetic centers: Ceahlău, Ciucaș, Bucegi, Lotru and Apuseni. Romanian larch belongs to the *Carpathian* subspecies and is an ecotype of altitude, grows over 1200 meters altitude with Swiss pine and Norway spruce of altitude.

The speed of growth, high productivity, high wood quality and resistance to adversities led to the initiation of researches on larch breeding. Twenty-one clone orchards totalizing 119 hectares have been established between 1964 to 1982. More than 500 plus trees were selected both from natural and artificial stands, and first - generation seed orchards represent now a broad genetic base for breeding. Also, in recent years due to windfall widely occurring in pure spruce stands, the percent of larch has increased in reforestations. Most of the forest reproductive material comes from seed orchards.

Since 2007 an advanced – generation breeding plan for European larch was started. In order to evaluate the best parents from first – generation seed orchards based on their genetic values a half-diallel mating design was used. Information on general combining abilities (GCA) of parents and specific combining abilities (SCA) of progenies will be used for future selection and breeding. In addition, to compare expected genetic gain from variety designs, open – pollinated seeds from the same parents were collected from the seed orchard in the same year. 43 full-sib families and 29 half-sib families were obtained by now.

This paper presents results from a 10 x 10 half-diallel and one half-sib experiment of European larch at the age of 2, 3 and 4 in nursery. Researches focused on assessment of the additive and dominance genetic variances, heritabilities, genetic correlations and expected genetic gain for phenologic, growth and branches characters.

At the nursery stage, the variance analysis reveal a very significant differences ($p < 0.001$) among parents and families in both experiments. General combining ability (GCA) and specific combining ability (SCA) are very important sources of variation for the studied characters. Dominance variance (σ^2_{SCA}) exerted a greater influence as evidenced the $\sigma^2_{SCA} / \sigma^2_{GCA}$ ratios: 5.8 for root collar diameter and 6.3 for total height. Broad - sense heritability and genetic gain are higher in full – sib experiment comparative with half- sib experiment. Narrow - sense heritability is very small, ranged within 0.09 to 0.22 but was increasing over ages. GCA and SCA effects were calculated for studied traits indicate that selection for second-generation breeding and seed orchard program could be done at family level as well as at parents' level.

Key words: larch, additive variance, non-additive variance, combining ability, heritability, genetic gain.



Seed Orchard of Warm-temperate Tree Species in Southern Korea

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Abstract: Seed orchards of warm-temperate species are located in Jeju Island (Warm-temperate Forest Research Center, southernmost area of the Korean Peninsula). Total area of seed orchards is 156.2 ha. Our seed orchards have been set up since 1969 and consist of six coniferous species and 20 hardwood species. There are a few clone banks of *Chamaecyparis obtusa* (Japanese cypress or hinoki, 46.0ha), and *Cryptomeria japonica* (Japanese cedar or sugi, 30.0ha). The major species of our seed orchard are those hinoki and sugi species and the seeds of two species have been produced only in our orchards from late 1970s. Hinoki has been used for fine-quality furniture, musical instruments and there has been a sharp rise in demand because of the effect of the eco(forest)-healing. The production of hinoki has been much increased from 1989 (158kg), the maximum production of hinoki seeds amounted to 2,100kg in 2001. The average production of those is about 160kg every year and the good harvest of hinoki seeds has been came every fourth year. Sugi had been mainly used as a windbreak forest for mandarin plantations in Jeju Island, but use of sugi has been decreased. The average production of sugi seeds is about 65kg every year and the amount of cycle was not regular. We report research results from several trials for the production of more (in quantity) and superior (in quality) seeds from the seed orchards, by means of the application of genetic thinning, the analysis of flowering variation, the consideration of climatic conditions, and so forth.



Overview of Commercial Seed Production Systems of Temperate and Sub-tropical Eucalypt, Pine and Acacia Species in South Africa

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Abstract: The annual demand for genetically improved planting stock exceeds 200 million seedlings and cuttings per year. The bulk of the demand is made up of seedlings of various temperate and sub-tropical eucalypt, pine and acacia species and the remainder are hybrid cuttings of both eucalypt and pine species. The current commercial species have been developed for specific niche markets and site requirements within the landscape. Production of commercial quantities of seed is a key step in translating breeding and development into realised gains in field operations for the traits of interest.

A range of orchard types such as breeding seedling seed orchards (BSO), seedling seed orchards (SSO) and clonal seed orchards (CSO) have been developed to produce not only open pollinated seed but also allow for the production of control pollinated seed. Each orchard type and combination of superior genotypes will deliver a specific seed product with varying levels of improvement. In addition associated technologies for the production of control pollinated seed have been developed for the advancement of both eucalypt and pine cuttings programmes. Species of importance in the South African context are *Eucalyptus grandis*, *E. dunnii*, *E. nitens*, *E. macarthurii*, *E. smithii*, *Pinus patula*, *P. elliottii*, *P. taeda* and *P. greggii* and to a lesser extent *E. benthamii*, *E. urophylla*, *P. tecunumanii*, *P. caribaea* and *Acacia mearnsii*.

The key focus areas in the development of improved seed are:

- Developing an understanding of the breeding systems of each genus within specific environments to facilitate and enhance seed production.

- Developing orchard technologies for sustained and enhanced production.
- Management of gain from the orchard through to field establishment.
- Developing seed processing technologies and new seed products.

Key words: *Eucalyptus*, seed orchards, superior genotypes, gain.



Genetic Consequences of Utilization Artificial Populations in Forestry

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Abstract: Breeding of forest tree species can be described as taking genotypes from their wild, undomesticated state through a selection, with the potential of reducing genetic variation and in the same time resulting in phenotypical superior characters. In this paper, we review some genetic diversity implications of different selection effects on silviculture and breeding of *Quercus robur*. Genetic study revealed loss of genetic variation and adaptive potential within seed orchard and its seed crop. Therefore it would be well advised to utilize of breded seed crop in the best possible site condition. It would provide the most effective utilization of expected genetic gain as well.

Key words: *Quercus robur*, selection, seed orchard, seed crop, genetic diversity.

Introduction: The designation of seed stands of higher quality was started in Hungary at the end of the 1950's, and these selected stands have been the basis of qualitative seed production to this day. Besides mass selection also more intensive breeding activity started in order to improve economically valuable, generatively propagated tree species. Seed orchards have been established from the descendents or grafts of high timber quality plus trees assigned on the field selection. The two main characteristics of seed plantations are the defined combination and ratio of clones or progenies of known origin and the purpose of seed production usually with the aim of higher quality (El-Kassaby 1999, Hosius et al. 2006). Several important questions arise in connection with the genetic background of seed crop produced in orchards considering the utilization and the future breeding aims, namely

What rate of genetic diversity can be held by an artificial population composed by a few clones in comparison with natural stands?

- How will this diversity change in the next generation during seed production or does it change at all?
- What is the exterior pollen ratio coming from outside the plantation and how does it affect the genetic composition of seed crop?
- In conclusion of all the information above, on which sites is the utilization of seed crop produced in orchards recommendable?

These questions are especially important to answer due to the fact that tree species with extrem long life-cycle bear much higher genetic diversity than is necessary in the current ecological conditions for the actual survival (*Müller-Starck* 1995). This 'excess' is the raw material for adaptability, and involves the future possibility of continuity in case of drastic environmental changes or appearance of new pests or phatogens (*Ziehe et al.* 1999).

Material and Method: The study plot is a pedunculate oak (*Quercus robur*) seed orchard (Sellye, Mecsek Forestry Corporation) containing drafts of 56 genotypes that were selected with high timber quality from the Mecsek Mountain, Southwest-Hungary. Dormant winter buds were collected for genetic analyses from the 56 mother genotypes and from 437 seedlings originated from the plantage. Furthermore, a native pedunculate oak stand was selected close to the plantation (3 km) where a long term field trial was established in 1962 by the Hungarian Forest Research Institute in order to evaluate the consequences of different thinning methods (*Birck et al.* 1962). 50-50 individuals from three plots (intensive thinning, normal thinning, control plot) were randomly collected and added to the analyses for comparison.

Izoenzyme study were carried out analysing the following enzyme systems: AAT-B, ADH-A, AP-B, EST-A, GDH-A, IDH-B, MNR-A, PGI-B, PGM-A, SKDH-A, 6PGDH (*Zanetto et al.* 1996). Moreover, four higly variable nuclear microsatellit loci (ZAG 1/5, ZAG 15, ZAG 20, MSQ 13) were applied in order to estimate the ratio of pollen flow in the plantation.

Results and Discussion: 56 adult tree genotypes from a pedunculate oak seed orchard and 437 seedling genotypes was analysed in order to detect the occurrent change in the genetic composition of the produced seeds. One of the most apparent result of the study is the emergence of new alleles in the second generation (seed crop) compared to the parent trees in the plantation. The mean number of observed alleles per locus (N_a) increased comparing the subpopulation of adult trees in the plantation with the progenies from 3,36 to 4,45 in case of the

isozymes and from 9,00 to 11,25 in case of the four microsatellite loci (Table 1). This is the most obvious evidence of 'foreign' – exterior pollination.

However the occurrence of external pollination has rather a rare and negligible effect if we consider the effective allele numbers (N_e). These values are weighted with the frequencies of alleles, and therefore the dominance of a few pollinator individuals in the orchard is more probable. In case of the human affected plots, the decreasing tendency of the N_e values indicates the selection pressure of the more and more intensive thinnings (1,70 – 1,64 – 16,61). This impact is true even if the selection – positive tree selection or thinning – is based on phenotypic characteristics. Along with the assignment of valuable phenotypes a certain genetic pattern would be preferred even if it was not intended during the fieldwork.

Heterozygosity values show an analogous trend, that is the more intensive the selection procedure was the lower diversity could be observed. This affects the adaptability disadvantageously, therefore the subpopulation of progenies would have a lower chance to adapt in a varying, climatically stressed environment due to unfavourable temperature or precipitation conditions.

The genetic relationship was represented on an UPGMA dendrogram where the Nei's genetic distance of the 5 subpopulations was plotted based on the genetic data given by the 11 isozyme loci (Figure 1). It can be clearly seen that the subpopulations of the orchard and the seed crop produced there have a distinct position compared to the near forest stand plots. We can conclude, that the progeny generation produced from the plantation is less natural. From the results it is also observable, that the moderate and expertly executed stem number reduction (thinning or cleaning) caused a less vigorous decline in the genetic diversity. At the same time an intensive intervention would have an adverse effect on the further adaptability of the remaining population.

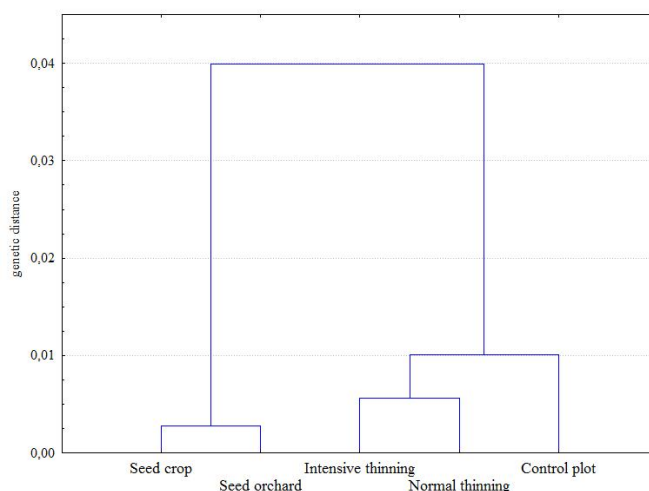


Figure 1. The genetic relationship of the subpopulations based on the Nei's genetic distance.

Intensive selection processes (reduced genetic diversity) are observable both in case of the selected plus trees and their progenies, while the exterior pollen amount was not sufficient to improve diversity. From this result the conclusion can be drawn that seeds produced in the orchard carry a lower adaptability potential as a second generation, however – on the other hand – the breeding aim, namely crossing trees of outstanding quality certainly succeeded.

Therefore the utilization of orchard seed crop is recommendable on the best sites, where the ecological stress is the lowest and the reduced adaptability would not hinder the sustainability of stands (*Gram et Sork 2000*). Moreover the expected breeding gain by crossing higher quality individuals can be better exploited and realized on better sites as well (*Finkeldey et Ziehe 2004*).

The results presented here also point out that the qualified thinning methods with moderate stem number reduction would not necessarily cause more aberration in the genetic pattern of oak stands than the normal natural processes.

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Table 1. The main genetic parameters of the analysed subpopulations.

	Control plot	Normal thinning	Intensive thinning	Orchard	Progenies
11 isozyme loci					
N_a^*	4,09	3,45	3,55	3,36	4,45
N_e	1,70	1,64	1,61	1,56	1,55
H_{obs}	0,25	0,24	0,22	0,20	0,18
H_{exp}	0,38	0,36	0,35	0,29	0,28
4 microsatellite loci					
N_a	-	-	-	9,00	11,25
N_e	-	-	-	4,32	4,10
H_{obs}	-	-	-	0,75	0,65
H_{exp}	-	-	-	0,75	0,72

*; N_a – number of observed alleles; N_e – number of effective alleles; H_{obs} – observed heterozygosity; H_{exp} – expected heterozygosity; all mean across loci.



Optimal Use of Genetics in Deployment Using SEEDPLAN Tools

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Abstract: The objective of a tree improvement program is to breed, select and deploy genetic material with improved biological characteristics for traits of commercial importance.

The Southern Tree Breeding Association (STBA) and its partners work with various tree species grown for different production environments and processing systems. A large amount of biological data has been collected on trees over decades of breeding in Australia for the main commercial species of *Pinus radiata*, *Eucalyptus globulus* and *E. nitens*. The national cooperative (STBA) uses its web based DATAPLAN system to manage the data and information, as well as facilitate access by breeders, researchers and industry personnel. The TREEPLAN system is used for the genetic analysis of tree breeding data on a program, species wide or global basis. All performance data collected in hundreds of trials over time is combined as part of national genetic evaluations using full pedigree. The resultant genetic values for selection criteria (measured traits) and commercially important breeding objective traits allows for the objective comparison of trees and genetic material for breeding and deployment. Results are reported on a regional basis, to ensure genotype by environment interactions are accounted for and the best genetics is identified for each situation.

Making optimal use of genetics in deployment systems can be problematic given the large amounts of information (genetic, economic and inventory) to consider and the range of genetic material (seed and plants) available at any given time. As part of the SEEDPLAN project, we are developing a suite of software tools that will assist the breeder and forest grower in the optimal use of genetics when

developing orchards and seed lots for specific combinations of environments and processing systems.

A selection tool, SELECT, is designed for selecting genotypes for grafting into breeding arboreta or deployment orchards for seed production. Placing limits on group co-ancestry and determining the optimal contributions from individual genotypes are major considerations, while monitoring trends in reproductive and fitness traits is also considered. The MATE allocation module is used to determine what crosses are best for maximising gain for a particular objective, subject to constraints associated with potential inbreeding. Design of seed orchards and the optimal placement of ramets to avoid mating among close relatives are done using the DESIGN module. Determining the genetic composition of seed harvested from trees in an open pollinated orchard with the COMPOSE module needs to account for census information and dynamics of pollen dispersion. Companies can use the INDEX module to refine their economic weights for important traits in their deployment objectives, depending on the particular target environment and production system under consideration. The MATCH module allocates seed and plant lots to stand types in an optimal manner to maximise enterprise profitability.

In developing software tools to aid decision making, it is important they be flexible to cater for the different needs of the many users dealing with various species and business environments. It is also important the modules are integrated within the overall framework of tools for managing data and information flows.

Key words: tree breeding, deployment, genetic improvement, economic worth.



Reduced Background Pollination by Isolation?

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Background: Forest tree breeding in Sweden has reached a level where we establish orchards with genetically tested and selected first generation plustrees for both Scots pine (*Pinus sylvestris* L.) and Norwegian spruce (*Picea abies* (L.)). The genetic gain of these selected first generation plustrees is approx. 25 % on average (Rosvall *et al.* 2001).

To increase seed quality and crop, seed orchards for northern Sweden are usually established at milder locations and at lower latitudes than their corresponding clone origins. Background pollination from surrounding stands will then not only decrease genetic gain but can also cause maladaptation, since southern pollen sources are less hardy (Persson & Ståhl 1993, Persson 1994). Further, the background pollination in mature Scots pine seed orchard is estimated to be at least 40-50% (e.g. Eriksson 1996, Torimaru *et al.* 2009), and thus significantly impacting growth and adaptation of orchard seedlings. Minimizing background pollination is therefore of great concern for Swedish forestry

In this study we investigate the possibility to decrease the background pollination by isolating the trees in greenhouse during bud flush and flowering. The idea is that trees in greenhouses flowers earlier and more intensively than surrounding trees. The greenhouse has also the function of a shelter against background pollination.

Key words: background pollination, isolation, tunnels, microsatellite, seed orchard, Scots pine.

Material and methods: The experiment is performed in seed orchard FP-621 Västerhus (lat. 63,3° N, 15 m.a.s.l.) established in 1991 with 28 clones of mean origin lat. 63,8° N 308 m.a.s.l. To minimize grouping of clones, the clones in the orchard were divided in three groups with one of the groups planted in every third row.

In an earlier study from the cone crop 2007 in Västerhus using microsatellites, the pollen contamination was estimated to be 47% (Torimaru et al 2009, 2011). The pollen production 2006 (the year of pollination) was estimated to 27 kg/ha.

In the experiment we have six isolation greenhouses (tunnels), each 30,8 x 7,0 x 5,5 m (length, width and ridge height). The tunnels are built with steel pipes as carrying construction. The tunnels has 2,5 m high walls along the sides. The walls are joined together with 10 m long steel pipes, bent to 7 m broad bow between the walls. On top of the bows, along the walls and at the ends, the tunnels are covered with polythene plastic. During extremely warm days the tunnels can be opened up in the ends to keep the temperature down, however, in Sweden, that's rarely the problem in springtime. It is also possible to lift up the walls to ventilate along the side.

In each tunnel there are 12-14 grafts. All graft in all treatments comes from the same group of clones that consist of ten of the 28 clones that are represented in the orchard. In two tunnels we do nothing but isolate the grafts. In two tunnels we blow around the pollen within the tunnel with a blower. In the last two tunnels we add pollen from five highly ranked clones in the orchard that are not represented in the tunnels. We also have two control plots with no tunnel and no other treatment. All treatments are aggregated in the mid part of the orchard, replicated twice and randomly distributed within two blocks, table 1. As we noted that a tunnel can look like a "steam engine" during pollen shed and might spill over pollen to early flowering trees in controls, therefore we added three supplemental controls in other parts of the orchard as control to the controls.

Table 1. Treatments with each two replicates, except for treatment no 5 that has three replicates.

1. Isolation in tent
2. Isolation in tent + blower to increase pollination success
3. Isolation in tent + supplemental pollination to increase pollination success
4. Control plot
5. Supplemental control in other parts of the orchard

The trees were isolated from mid-May to mid-June in 2010 and 2011. Pollination within tunnels was completed in early June. To minimize the risk of frost damages,

the plastic sheet was kept on until mid-June. We plan to repeat the experiment in 2012 as well to capture yearly variations.

All cones produced in tents, have been and plan to be collected from individual trees in 2011, 2012, and 2013. We also collected the cones from 2010 as a bulk of each replicate.

The 28 clones within Västerhus have been mapped using microsatellite genotyping in an earlier study (Torimaru et al. 2009). Using the same technique the background pollination and the father's contribution will be estimated for seed from individual grafts from the different treatments. In parallel to the genetic analysis we will also analyze whether the crop size and seed quality are influenced by the treatments.

We also have two pollen collectors within the orchard and two collectors 400 m outside of the orchard with daily sampling during pollen shed.

Fenology studies of male and female flower development and receptiveness in four clones that are represented in all traits were recorded in tunnels and control.

Pollen production per graft was and plan to be recorded in 2010, 2011 and 2012 for all trees in the experiment and in two sample plots in the orchard. The number of male strobili was counted on half the tree and the mean strobili length was estimated. The total strobili length in meter per tree was multiplied with 0,00028 (Koski 1975).

Results: Building tunnels with 2,5 m high walls was new, and not testes according to our supplier. After two years testing, the tunnels seem to withstand quite hard weather conditions. In a nearby climate station (Järved) gusts up to 19 m/s was measured in early June 2011.

Male and female flowerig: We seem to succeed in separating the female flower receptivity from the pollen shed of surrounding trees, figure 1. We also seem to make the pollination period more intense and less independent of outdoor weather conditions. In ordinary, open air orchards, the pollination may be interrupted because of cold and/or wet weather, however, using tunnels the pollination can proceed even during less favorable weather conditions.

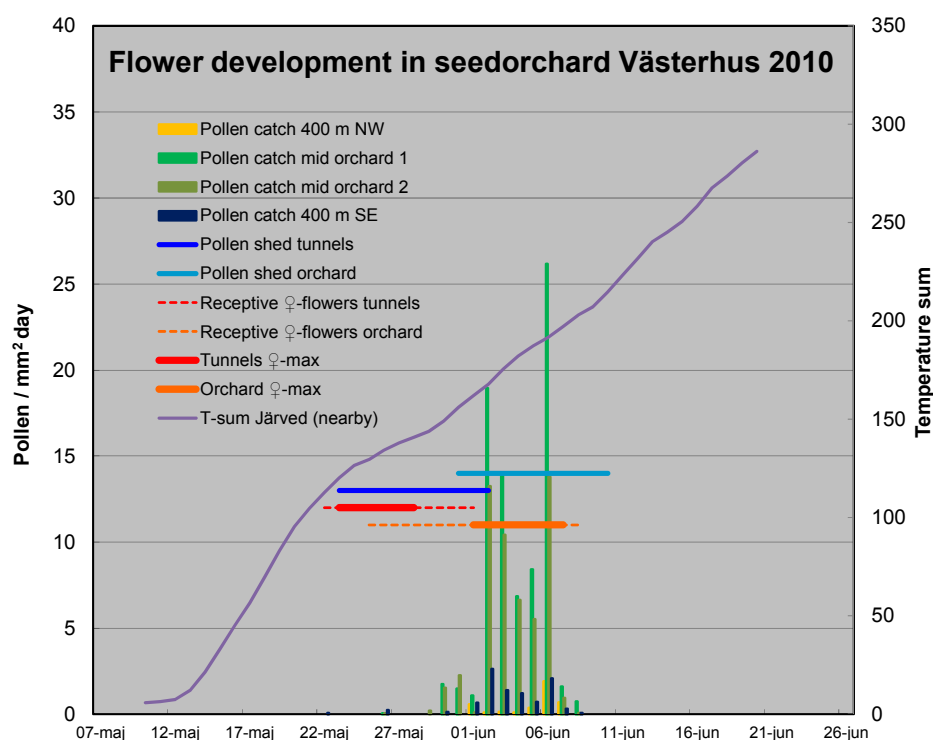


Figure 1. Male and female development in tunnels and open air, results from pollen catches, fenology studies and temperature sum in seed orchard Västerhus in year 2010.

Seed crop and quality: Seed from the cone crop 2010, pollinated in open air in 2009 but started their maturation process of seeds in 2010 with four weeks in the tunnels, had 10% higher seed weight than control ($p=0,0064$), table 2. Other germination properties testes were similar to control.

Table 2. Number of seeds per graft, germination capacity after 14 days (G14), germination energy (GE) and weight of 1000 seeds (1000-sw). Results from germination analysis from cone crop 2010.

	Seeds/graft	G14	GE*	1000-sw
Tunnels	1869	98,2	97,1	7,30
Control	2007	98,1	98,5	6,66

*, GE is calculated as number of germinants after 7 days divided with the final number after 14 days at Jacobsens apparatus at 20°C 16 h/day and 30°C at 8 h/day at constant light on 4 x 100 seeds.

So far we have not seen any sign of frost damages on flowers, shoots or seed.

Microsatellite mapping: Since the experiment started in May 2010 and we collected the cones in October 2011 we do not have any results from the microsatellite mapping yet.

Discussion: It is possible to enlarge the experimental tunnels to full scale tunnels that cover a whole orchard. The tunnels can be 500 m long, containing one whole row of trees in an orchard. It is also possible to take away the walls between rows and allow pollen to migrate to a certain extent between rows as well as within rows. When we have final results from the experiment it is possible to compare the value of increased genetic gain (and seed amount and quality) with the cost for tunnel treatment, on a full operational scale.

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Back in Turkey: Breeding Without Breeding: Minimum Genotyping Effort

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Abstract: The term “Breeding without Breeding, BwB” has been coined by El-Kassaby et al. (2006) in Turkey as a relatively simple breeding concept. During the past five years, it has been recognized in tree breeding community as a competitive stand-alone tree improvement scheme with many applications worldwide. BwB is based on natural pollination, thus expensive and laborious artificial crosses are eliminated, followed by pedigree reconstruction and simplified testing arrangements. This is appealing in many current tree improvement programs where resources have been restrictive. It has been demonstrated, that BwB strategies are fully competitive, in terms of capturing gains attributable to classical breeding strategies (El-Kassaby and Lstibůrek 2009, El-Kassaby et al. 2011).

In our investigation, we have acknowledged causative variables to the BwB's efficiency concomitant to the genotyping effort. These include: genetic response to selection, maintenance of satisfactory levels of gene diversity, and minimum sample size compulsory to efficient pedigree reconstruction. In our presentation, we will discuss our latest mathematical protocol, based on the graph theory, to minimize the genotyping effort (Lstibůrek et al. 2011). We will highlight the relevance of this theoretical work to minimizing the fingerprinting cost in BwB applications. Our results indicate that fingerprinting small fraction of individuals is adequate to meeting all important requirements, thus making the BwB yet more tempting to the tree-breeding community.

Key words: Breeding-without-Breeding, Pedigree Reconstruction, Seed Orchards

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Analysis of Polygenic Traits and Establishment of Advance Generation Seed Orchard in *Dalbergia sissoo* Roxb

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Abstract: *Dalbergia sissoo* Roxb. is an important tree species which is extensively grown throughout India under various forestry and agro forestry programs. High degree of variation is reported due to differences in seed origin and/or climate. *Dalbergia* genus is named after the Swedish Botanist, Nicholas Dalberg and is a pantropical genus with 100 species distributed throughout world. Twenty seven species of the genus are represented in India, of which 15 are indigenous and 3 endemic (*Dalbergia congesta* and *D. gardneriana* to Nilgiris and *D. wattii* to Manipur (Thothathri, 1987). *Dalbergia sissoo* occurs in many parts of India up to 900 m in the sub-Himalayan tract and occasionally ascending to 1000 m. With ever increasing demand of the quality wood in wood based industries coupled with over exploitation and utilization of bigger and high-quality logs, has become a concern to the researchers. Therefore, the genetic improvement of the species has become inevitable. The selection of candidate plus trees (CPTs) was carried in both natural forests and plantations in the states of Haryana, Punjab, Rajasthan, Uttarakhand and Uttar Pradesh and in Nepal. The index method of selection was adopted to select the plus trees from the CPTs as per the methodology described by Cotterill and Dean (1990). In this manner, 150 plus trees were selected and propagated clonally to establish in the clone bank at the Forest Research Institute, Dehradun, India. On the basis of preliminary growth performance and disease incidences, a total of 48 clones were further selected for understanding genetic diversity. The selected genotypes were multiplied through softwood single nodal cuttings dip-smeared with rooting hormone of Indole-Butyric Acid (IBA) with 2000 ppm. The well developed clones were field planted at two geographical locations of Hissar (N 29°30'17.7" E 75° 33' 14") in Haryana and Hoshiarpur (N31°33'31.7" E 75°49'0.5") to analyze genotype X environment (G X E) interaction among the selected clones. The trails were established following lattice design with six replication and two

ramets in each replication. The quantitative parametric traits viz. height and collar diameter were recorded periodically. At the age of eighteen months, mean pooled height ranged from 41.17 cm (clone 94) to 112.52cm (clone 5003) and the mean pooled collar diameter ranged from 0.59 cm (clone 31) to 2.02 cm (clone 5006) for both the locations. Mean value based on quantitative traits expressed superiority of thirteen clones over the locations. Divergence analysis was performed to identify the diverse genotypes for hybridization purposes in the seed orchards. Clustering using D^2 statics has been one of the potent techniques of assessing genetic divergence in which clusters separated by the largest statistical distance (D^2) show the maximum divergence (Singh *et al.*, 1985). In addition to aiding in the selection of the divergent parents for hybridization, D^2 statics measures the degree of diversification and determines the relative proportion of each component characters to the total divergence. Data therefore collected for growth traits of the selected forty eight genotypes of *Dalbergia sissoo* clones were subjected for genetic divergence. In this way, forty eight (48) clones were grouped into nine clusters and measured the differentiation among the clones at intracluster and intercluster levels. Among different clusters, cluster VIII consisted single clone (Clone 05) originated from Sabalgarh, Haridwar, Uttarakhand. The maximum D^2 value (1.62) was found between clones 1003 (Uttarakhand) and 5007 (Uttarakhand). The intra-cluster distances ranged from 0.00 (cluster VIII) to 0.06 (cluster VII) which was an indicator of substantial genetic diversity among the clones. Similarly inter cluster values ranged from 0.03 (cluster VIII & I) to 0.86 (cluster IX, II & V). Cluster IX was found to be the most divergent cluster with maximum values among the clusters followed by cluster VII. The inter cluster distances of cluster IX with other clusters was found to be maximum at six times (0.55 cluster I, 0.86 cluster II, 0.46 cluster IV, 0.86 cluster V, 1.33 cluster VII and 0.76 cluster VIII). The cluster means for growth characters under study revealed considerable genetic differences between the groups. Cluster VII, with six clones had the maximum mean value for height (57.83 cm) which also attained the second position as the most divergent cluster on the basis of D^2 values. Among different clusters, Cluster VIII consisted one clone had highest means value for collar diameter (0.79 cm). The situation indicated that the clusters VII and VIII were the most productive clusters which could play significant role in the genetic improvement when established appropriately in seed orchards. Clones assembled in the cluster IX were though found to be divergent, had minimum value for both the traits. It was therefore obvious that clones 05, 1003 and 5007 were the most divergent clones. It is emphasized that if such divergent clones are allowed planted to-gather in the

clonal seed orchards or interbred manually, there would be strong possibility for the exhibition of high degree of heterosis. In fact, it could play an important role in the production of new recombinants with maximum acquisition of traits of economic importance.

Key words: Cluster analysis, G X E interaction, quantitative traits, genetic diversity, heterosis.



Breeding and Genomics of Circum-Mediterranean Conifers

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Abstract: Conifers are keystone species of natural Mediterranean ecosystems. They represent valuable and often unique genetic resources, as they include endemic species and marginal or disjunct populations bearing specific adaptations. Since antiquity, Mediterranean conifers face since antiquity challenges related to their very existence, such as population decline, intensive exploitation, fragmentation, wild fires, pests and diseases. Climate change is an additional overarching pressure that may aggravate threats to Mediterranean forests, such as water depletion, new lethal pests and diseases, pollution and desertification. This is especially relevant considering that the Mediterranean basin is a major biodiversity hotspot. It is thus crucial to assess the existing genetic variation among and within populations of Mediterranean conifers, as well as to assess their adaptive, productive and thus, breeding potential for future sustainable use in reforestation programs and production plantations. Breeding efforts depend to a large extent on efforts at the national level. Circum-Mediterranean breeding efforts were initiated in the early 1970s and were co-ordinated by IUFRO (WG 2.02.13) and FAO-Silva Mediterranea. In this framework, many Mediterranean countries participated in the exchange of genetic material and the establishment of provenance and progeny trials to identify the seed sources with adaptive and productive potential for their local environmental conditions. The above network of trials for each Mediterranean conifer appears now in the database 'Comparative Trials of Mediterranean Conifers' hosted by INRA (Avignon)-France (<http://www.avignon.inra.fr/ForSilvaMed>). More recently, genomic approaches and new sets of quantitative traits have summed up to the collective effort of describing and understanding adaptive variation in Mediterranean conifers. The state of the

art of research regarding genomics and breeding of Mediterranean Conifers, as well as results from the circum-Mediterranean network of trials are reported, based on the up to date published works.

Key words: Circum-Mediterranean network of trials, adaptive potential, spatial genetic variation, provenance/progeny tests.



Clonal and Annual Variation in Fruit and Seedling Production from Seed Orchard and Other Seed Sources of Teak (*Tectona grandis* L. f.) in Southern India

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Abstract: Teak is a high priority timber species in India both as a major constituent of its deciduous forests and a popular plantation crop. India has the largest extent of teak-bearing natural forests and plantations in the world and is also referred to as the centre of diversity for the species. Since extraction from natural forests is banned, plantations are the main source of teak timber in India and the current production is not sufficient to meet the steadily increasing demand. India is one of the largest importers of teak and other tropical timbers in the world. The huge demand for teak timber has also resulted in increased investments in teak planting and genetic improvement activities.

India started teak improvement research more than half a century ago with the establishment of clonal seed orchards developed through grafts of phenotypic selections for growth, stem form and apparent freedom from pests. India has around 1000 ha of seed orchards of teak established over a period of three decades aimed at meeting at least part of the demand for genetically improved seed. Unfortunately most of these orchards did not function well to meet the seed demand for raising new plantations. The common problems faced in teak orchards were lack of flowering, asynchronous flowering among clones and pollinator limitation leading to low fruit and seed setting. Many studies have been conducted on teak orchards during the last decade generating new information to decide on retaining these orchards. We present here the dynamics in a teak clonal seed

orchard for seed and seedling production attributes and discuss its implications for future breeding and propagation programmes.

Fruit, seed filling, germination and seedling traits of teak were studied for three years in a clonal seed orchard (CSO), five seed production areas (SPA) and a natural population (NP). The orchard was established at Walayar, Kerala State (10° 50' N; 76° 50' E) in 1976 with around 475 grafts of 20 clones. SPAs were around 40-year old plantations with higher proportion of trees with desirable traits and harder thinned than regular wood-producing plantations.

Highly significant ($p < 0.001$) differences were observed for fruit size, weight and number of seeds per fruit among the different sources in all years of study. The NP had the largest and heaviest fruit and the CSO fruits were average or below average in size and weight. Teak fruit is a drupe with 4 locules and seed filling ranges from 0 to 4. The number of seeds per fruit was around 1 for CSO comparable to the overall mean for all sources. Fruit size and weight showed moderate positive correlation with seed filling.

Germination and seedling recovery (teak drupe is the unit of sowing in the nursery) showed highly significant differences ($p < 0.001$) among various sources in all years of observation. Germination ranged from 5 to 62% and seedling recovery 7 to 83%. Two SPAs (Cherupuzha and Sankarankodu) consistently showed higher germination (49 to 62%) and seedling recovery (59 to 83%) than all other sources. CSO showed poor germination in all years (5 to 20%). Clones in the CSO showed significant levels of variation ($p \leq 0.006$) for fruit, germination and seedling traits. A few clones of CSO showed comparable germination to the best SPAs only in the abundant flowering year. The magnitude of variation for germination was greater in CSO than SPAs across years.

A general lack of flowering appears to be the basic problem in teak seed orchard which in turn affect fruit production, seed set, germination and seedling vigour. During a 4-year period only once the proportion of flowering trees exceeded one third mark in the orchard and ranged from 17 to 28% in other years (Nicodemus et al. 2008). Contrastingly 62 to 96% of trees were flowering in SPAs and NP. Promoting the flowering abundance in teak orchards should be a priority in near future through ground management, imposing treatments like application of flower inducing plant growth regulators and culling consistently non-flowering trees to create space for fertile accessions. Alternatively seed collection may be restricted to the abundant seed years. New orchards may be located in areas known to be favouring good flowering.

While some of the options may be cost-prohibitive, most of them may not contribute to seed production in the immediate future. Further considering the inherent tendency of teak for low flowering and complex plant-pollinator interactions leading to low fruit and seed production, large number of trees is needed to produce the required amount of seeds with acceptable levels of genetic quality. Abundant seed production with adequate diversity is best achieved with low input breeding options like SPAs (Lindgren and Wei, 2007). SPAs can be a source of reasonably improved seed for current planting and also for developing next generation orchards (Kjaer et al. 1999). The large number of parent trees in SPAs ensures that high level of diversity is maintained in the progeny even if the gains are modest. SPAs are timber producing areas and the seeds can be seen as a side product, while clonal seed orchards are established to get seeds only. The economy of SPAs may be much better, and it is easier to defend their establishment in spite of a limited flowering at certain locations.

Key words: clone, flowering, germination, seed production area, seed set, variation.

Acknowledgements

This study was funded by International Foundation for Science (IFS), Stockholm through a research grant to A. Nicodemus (Grant No.D/3384-1). Assistance and field support was provided by the forest departments of Kerala and Tamil Nadu States to collect seeds from SPAs. R. Natarajan, A.M. Kumar, G. Ponraj, S.P. Subramani and K. Karthikeyan provided technical assistance in seed collection and recording germination data.

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Progeny Testing of Selected Clones of Eucalyptus for Growth Performance

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Abstract: Progeny testing plays a very important part in tree breeding. Progenies provide valuable information which are of economic value than the parent phenotypes. Progeny trials are conducted for the selected superior clones to estimate their genetic potential. Generally clonal trials are established with selected clones and after evaluation, these trials are converted to into Clonal Seed Orchard (CSO) to save cost and time involved in tree breeding. The spacing and the proportion of the genotypes present in the clonal trials are altered through culling for conversion into CSOs. Culling of poor clones or ramets within plot also depends on breeding system of a species.

Eucalypts are predominantly outcrossed species, pollinated by insects. Although random mating is possible through insects, related trees need to be avoided for high level of cross pollination. Clonal trials are established with more than 80-100 clones with three ramets per plot by adopting a spacing similar to that of routine plantation (3 x 2 m). About 1666 trees/ha. are planted in Clonal trials against the final spacing of 6 x 6 meters (250 trees/ha.) in CSOs. In general, reducing the stocking in orchards indirectly favours random mating.

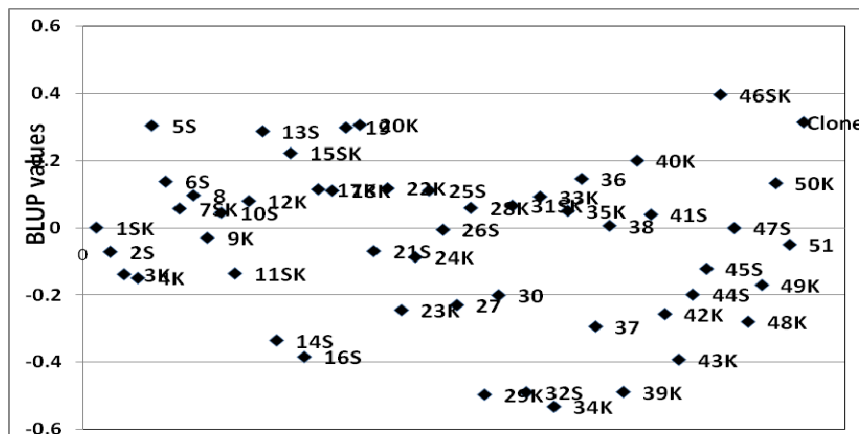
To study the progeny growth performance of seeds collected from the Clonal trial and to quantify the effect of increased spacing created by random removal of trees in clonal trial, two clonal trials were selected at Karunya (10° 55' N; 76° 44' E), Coimbatore, India and Sathyavedu (13° 28' N; 79° 51' E), Andhra Pradesh, India. Karunya and Sathyavedu were planted with 100 and 80 clones of *Eucalyptus camaldulensis* respectively during 2000. The Karunya trial was maintained with full stocking (1666 trees/ha) and in Sathyavedu trial trees were removed randomly

without any consideration for the merit of the clones with a stocking of about 300 trees/ha. Seeds were collected from the clones present in these Clonal trials and two progeny trials were established.

Progeny trials were established at Hyderabad (PT-1) (17° 18' N; 78° 25' E) with about 52 families of the clones. The family seed lots were made by collecting open pollinated seeds from the clonal trials. The trial was planted with 11 replications each with 3 plants per family. In another progeny trial established at Pudukottai (PT-2) (10° 22' N; 78° 42' E), the seeds were collected from the commonly replicated clones in both the clonal trials and were planted separately for comparison. The trial was planted with 3 replications each with 3 plants per family. After one year of plantation establishment, the height and girth were recorded.

Statistical analysis was carried out in PT-1 to find out the significant difference between the families. Standard error within each family was also calculated and given in Table 1. For the purpose of comparison, one of the selected clones was also planted along with the progeny. Volume was estimated from height and girth values and subjected to REML analysis. Family BLUP values were estimated and given in Figure 1. The clone and few families like 5S, 19, 46SK, 33K and 40K showed significantly higher growth performance when compared to other families. The family BLUP values also showed superiority of 46SK, 5S, 19, 20K, 13S and 15SK.

Figure 1. Family BLUP values for volume for selected *Eucalyptus camaldulensis* clones



In the PT-2, planted with seeds collected from 6 clones (1, 7, 11, 15, 31 and 46) commonly present in both the clonal trials. One year growth data was observed for the statistical analysis. Two way analysis was carried out. Progenies of thinned

clonal trial showed significantly higher growth performance than the un-thinned clonal trial (Table 2).

Table 1. Progeny growth performance of selected clones of *Eucalyptus camaldulensis*

Progeny	Height (m)		GBH (cm)		Progeny	Height (m)		GBH (cm)	
	Mean	SE	Mean	SE		Mean	SE	Mean	SE
1SK	3.09	0.12	7.28	0.49	27S	2.79	0.11	5.99	0.54
2S	2.99	0.09	7.29	0.50	28K	3.14	0.12	6.63	0.46
3K	2.89	0.13	6.65	0.59	29K	2.48	0.13	4.16	0.48
4K	2.92	0.11	6.52	0.49	30S	2.85	0.14	5.68	0.57
5S	3.36	0.09	7.95	0.41	31SK	3.11	0.11	6.69	0.49
6S	3.20	0.12	6.35	0.53	32S	2.53	0.12	5.28	0.53
7SK	3.11	0.11	7.28	0.48	33K	3.13	0.08	7.76	0.46
8K	3.15	0.13	7.28	0.58	34K	2.51	0.13	4.56	0.60
9K	3.03	0.11	7.13	0.51	35K	3.11	0.11	6.67	0.45
10S	3.09	0.11	6.83	0.41	36S	3.23	0.16	6.75	0.54
11SK	2.92	0.12	6.65	0.47	37K	2.76	0.13	5.59	0.52
12K	3.14	0.12	7.23	0.52	38K	3.09	0.11	7.53	0.53
13S	3.32	0.10	7.41	0.38	39K	2.56	0.14	4.39	0.47
14S	2.71	0.11	5.43	0.50	40K	3.25	0.13	7.56	0.62
15SK	3.27	0.14	6.90	0.48	41S	3.09	0.13	7.07	0.54
16S	2.64	0.10	5.83	0.49	42K	2.79	0.14	5.56	0.55
17K	3.16	0.12	7.31	0.43	43K	2.67	0.11	5.72	0.56
18K	3.13	0.09	6.86	0.40	44S	2.83	0.12	6.49	0.50
19K	3.35	0.14	7.83	0.57	45S	2.93	0.11	6.08	0.55
20K	3.30	0.12	6.91	0.40	46SK	3.43	0.12	7.52	0.52
21S	2.94	0.12	6.28	0.53	47S	3.05	0.12	7.20	0.52
22K	3.21	0.10	7.45	0.50	48K	2.87	0.11	5.42	0.52
23K	2.80	0.13	5.43	0.54	49K	2.91	0.16	7.10	0.66
24K	2.91	0.16	6.43	0.69	50K	3.17	0.11	7.30	0.47
25S	3.14	0.09	7.60	0.38	51K	2.99	0.15	6.85	0.53
26S	3.02	0.07	7.78	0.36	Clone	3.35	0.09	8.10	0.43
Mean	3.01	0.02	6.68	0.07					
SEd	0.135		0.550						
LSD	0.266		1.079						
CV%	21.2		39.1						

S- Sathyavedu; K- Karunya

The study reveals that clonal trials could be effectively converted into a viable CSO. High stocking in the clonal trial gives us scope for culling the poorly performing clones and retaining best performing clones. The number of ramets/ clones can also be altered keeping the final spacing required for efficient seed production in CSOs.

Table 2. Progeny growth attributes of seeds collected from un-thinned and thinned Clonal trials.

Family	Height (m)			GBH (cm)		
	Un-thinned Clonal trial	Thinned clonal trial	Mean	Un-thinned Clonal trial	Thinned clonal trial	Mean
1	3.89 (0.19)	4.27 (0.16)	4.08	8.92 (0.70)	11.33 (0.51)	10.13
7	4.22 (0.15)	3.70 (0.12)	3.96	10.58 (0.79)	9.83 (0.41)	10.21
11	2.64 (0.43)	4.43(0.19)	3.54	6.50 (1.20)	11.92 (0.65)	9.21
15	3.38 (0.33)	4.18 (0.15)	3.78	8.83 (1.11)	10.17 (0.55)	9.50
31	2.70 (0.38)	2.83 (0.45)	2.76	6.93 (0.81)	5.83 (1.22)	6.38
46	4.19 (0.26)	3.87 (0.19)	4.03	10.58 (0.94)	9.58 (0.65)	10.08
Mean	3.50 (0.14)	3.88 (0.11)	3.69	8.72 (0.42)	9.78 (0.36)	9.25
	SEd	LSD			SEd	LSD
Orchard	0.156	0.308		Orchard	0.454	0.897
Family	0.270	0.534		Family	0.786	1.554
Orc.Fam	0.382	0.756		Orc.Fam	1.111	2.198



Accelerating Tree Breeding Programs and the Impact on Forest Productivity and Profitability in the Southern US

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Abstract: Maintaining progress in tree improvement has huge economic development implications for the US South. Unlike other silvicultural inputs into plantations, the benefits from planting genetically improved seedlings are permanent and spread over millions of hectares at minimal extra cost. The economic incentive to increase efforts in tree breeding is impressive. If the genetic gain per year is increased to any extent, the regional financial impacts are worth millions of dollars. For example, the present value (6% interest rate) of a series of continuously improved plantations (1% per year of genetic improvement) was estimated to be \$4962 USD per planted hectare (e.g. a non-ending series of genetically better plantations of one hectare being planted each year). If these same plantations were established with the seedlings of only slightly higher genetic quality each year (i.e. genetic gain is increased from 1% per year to 1.1% per year), the present value would be \$97 USD per hectare planted per year. For the US South where about 500,000 hectares of loblolly pine are planted each year, the increased present value to all landowners from this slight increase in genetic improvement would be \$300 million USD.

The NC State University Cooperative Tree Improvement Program continuously works to discover innovative ways to accelerate tree breeding, while reducing costs and increasing efficiency. For example, our group has initiated tandem selection for fusiform rust resistance for elite breeding populations followed by clonal testing to select for growth and quality traits. We are testing new genomic tools for genome-wide estimated breeding values, and we are using markers to construct realized genomic relationship matrices to increase accuracies of breeding values to increase genetic gains. Our fourth cycle breeding strategy, now being developed, will incorporate opportunities to utilize genomic data.

Key words: Economics, genetic gain, *Pinus taeda*, tree improvement



Genetic Correlation between the Qualitative and Quantitative Traits of Plus Trees and Progenies of European Larch (*Larix decidua* Mill.) in Seedling Seed Orchards

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Abstract: The aim of the research was to determine the genetic variation in the growth and qualitative traits of the selected parent larch trees and their progeny in seedling seed orchards and the correlation between the analysed traits of parent trees and their progeny.

A total of 160 parent trees and their progeny growing in five seedling seed orchards were used in the experiment. Analyses were carried out for individual objects (orchards) and the tree groups formed on the basis of qualitative (A+, A, a) and quantitative (B, b) indices calculated for parent trees on the basis of measurements and observations. The variation in the progeny of trees was estimated on the basis of single measurements and evaluation of quantitative and qualitative traits.

The individual variability of parent and comparative larch trees was relatively high, especially with regard to quantitative traits. The differences reached up to 15 meters for height and 40 cm for breast height diameter. The variability of plus trees and comparative trees based on the mean values of analysed traits was definitely lower, but still very distinct. For the majority of the analysed traits, their values were higher for parent trees than for comparative trees. The parent larch trees were also clearly superior in terms of qualitative traits.

The regression and correlation coefficients calculated for the height of the parent and comparative larch trees were relatively high. The breast height diameter was in fact the only quantitative trait differentiating the parent and comparative trees.

The variability of quantitative and qualitative traits of larch families in the analysed seedling seed orchards was relatively high. In most cases, the differences were

statistically significant or highly significant – for breast height diameter, height, and seed production oscillating around 0.01, while for stem straightness and pilodyn penetration around 0.05.

Generally, the heritability for quantitative traits, both family and individual, was relatively high, though different on individual plots. At the family level, they assumed the value of up to 0.7-0.8, and at the individual level - even 0.4-0.5.

The value of correlation coefficients for the traits in the families in the analysed seedling seed orchards significantly differed, and usually was higher for quantitative traits, and lower for qualitative traits, while those of genetic correlations were frequently higher.

As regards seedling seed orchard groups the regression coefficients were usually relatively low, negative and not significant for any of the analysed traits. Also, the heritability coefficients were usually relatively low and had negative values.

Clearly different results were obtained for larch tree groups in terms of qualitative traits.

In the “A+” group of trees, the values of regression coefficients were markedly different. The regression coefficients were statistically significant for all quantitative traits. Although the values of the regression coefficients for qualitative traits and quantitative traits were comparable, the analysis did not show statistically significant differences between them. Also, the heritability coefficients calculated from the regression were comparatively high. In the case of height and breast height diameter, the heritability coefficients were close to unity and were statistically significant. Among the qualitative traits, the values of heritability coefficients were relatively high only for branch thickness (0.3988) and crown structure (0.32091), but their significance was not confirmed.

In the “A” group of larch trees, the regression coefficients were clearly different, assuming positive and negative values. For most quantitative traits, the regression coefficients were statistically significant. Although the values of regression coefficients for qualitative and quantitative traits were similar, the statistical analysis did not show statistically significant differences between any of them. The heritability calculated from the regression for the qualitative traits was also very high; in most cases it was statistically significant, but negative.

The regression coefficients for the “a” group of larch trees were clearly different, assuming positive and negative values. In the case of quantitative traits, however, the regression coefficients were not so distinct. The regression coefficient values were positive and statistically significant only for the breast height diameter. However, in the case of qualitative traits, the statistical analysis did not show

statistically significant differences between any of them. The heritability calculated from the regression for qualitative traits was also very low although in most cases it was statistically significant. Like in the case of regression coefficients, the calculated heritability for most traits was negative.

In the “B” group of larch trees, the regression coefficients were clearly different, in most cases assuming positive values for quantitative traits, and negative values for qualitative traits. Like in the “A+” group of trees, the regression coefficients for all quantitative traits were highly statistically significant (0.001). However, as in previous groups, the regression coefficients for qualitative traits were not statistically significant, assuming negative values in the majority of cases. The high values of the regression for quantitative traits resulted in the high heritability of these traits calculated from the regression.

In the “b” group of larch trees, the regression coefficients were significantly different, usually assuming positive values for quantitative traits and negative values for qualitative traits. For this set, the heritability values for quantitative traits were also relatively high. The heritability of these traits calculated from the regression was in most cases statistically significant. The heritability of qualitative traits were also relatively high, in most cases statistically significant, but negative.

Key words: larch, plus trees, seedling seed orchards, correlation, regression.



Genomic Selection in Forest Trees – Preliminary Results with *Pinus taeda*

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Abstract: Models using phenotype and resemblance among relatives have been successful in plant and animal breeding programs to increase genetic gains for complex traits. Many traits of interest to breeders are polygenic, being controlled by many genes each with small effect (Hill et al. 2010). These small-effect genes are crucial for the success of complex trait improvement (Crosbie et al. 2003).

Quantitative trait loci (QTL) mapping is important to explain gene architecture of complex traits. Unfortunately, QTL mapping and candidate gene approaches have had limited use in breeding programs. Compared with medical research, the emphasis in breeding is on predicting genetic merit of lines rather than on discovering individual genes. A good predictor of genetic merit does not have to identify the underlying genes (Goddard and Hayes 2009). What is needed a large number of markers to populate the genome and to explore the linkage disequilibrium (LD) between markers and many QTL with small effect. This approach is called genomic selection (GS) (Meuwissen et al. 2001).

GS contrasts greatly with traditional marker aided selection, because in GS there is not a defined subset of significant markers used for selection. Instead, GS jointly analyzes all markers in a population, attempting to explain the total genetic variance with dense genomewide marker coverage through summing marker effects to predict breeding values of individuals (Meuwissen et al. 2001). With advancement in DNA sequencing technologies and efficiency in genotyping, selection based on dense markers has become a reality in dairy cattle breeding (Goddard and Hayes 2007). Many livestock breeding programs now routinely apply genomic selection to market bulls (Hayes et al. 2009).

If successful, the impact of genomic selection on forest tree breeding could be far greater than for other crops or animal breeding programs, because forest trees take many years to breed and progeny test. Breeding programs are logistically

more complex and costly, and forest trees are still at the first stage of breeding with little genetic difference from natural populations. The few early empirical studies on genomic selection in forest trees are encouraging. For example, in a cloned *Pinus taeda* breeding population, accuracies of GS varied between 0.55-0.88, matching the accuracies achieved by conventional phenotypic selection (Resende et al. 2011). In an independent cloned population of *Pinus taeda*, Isik et al. (2011) also reported genomic estimated breeding values with reliability as high as breeding values based on resemblance among relatives and phenotypic data for a cloned progeny test.

There are different statistical methods to develop marker-based prediction models for GS. The effect of markers or haplotypes can be estimated by simultaneously by fitting them at once, using either BLUP or Bayesian approaches (Meuwissen et al 2001). Alternatively a smaller subset of markers can be used to estimate realized genomic relationships (G-matrix) using frequency of alleles shared by individuals (Legarra and Misztal 2008). Then, the additive genetic relationship matrix derived from pedigree is substituted by the G-matrix to predict genomic estimated breeding values. Genomic BLUP (GBLUP) could be a powerful tool for forest tree breeding programs. For example, Zapata et al. (2012) obtained higher accuracies of genomic estimated breeding values using GBLUP than using traditional pedigree-based BLUP methods. Such models can capture the Mendelian segregation effect in full-sib families, which was not the case using the average additive genetic relationships (Figure 1).

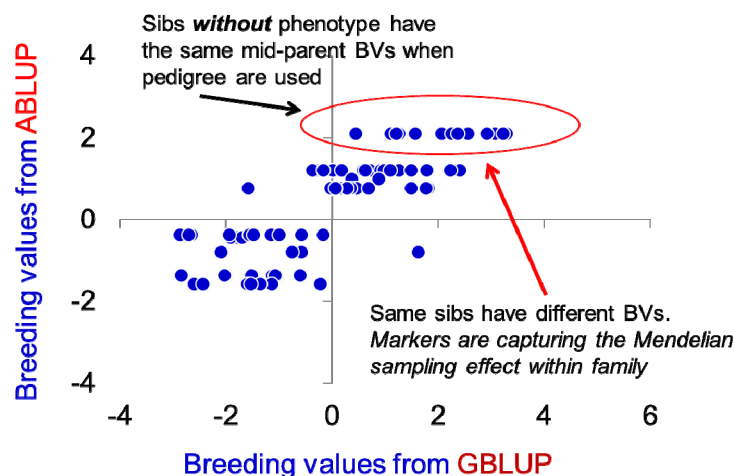


Figure 1. Predicted breeding values of *Pinus taeda* L. clones based on pedigree (y-axis) and based on genomic BLUP (x-axis). In the absence of phenotype, the expected breeding value of sibs is the mid-parent value. We cannot rank and make selections because all the sibs from a cross would have the same mid-parent BV. However, markers can capture Mendelian sampling effect as shown here, and thus, sibs can be ranked and selected without progeny testing (Zapata et al. 2012).

Genomic selection processes start from a training population (Figure 2). Candidates to establish a next cycle of breeding are selected through GS. While only a single occurrence of model training is shown, training can be performed iteratively as new phenotype and marker data accumulate (Heffner et al. 2011).

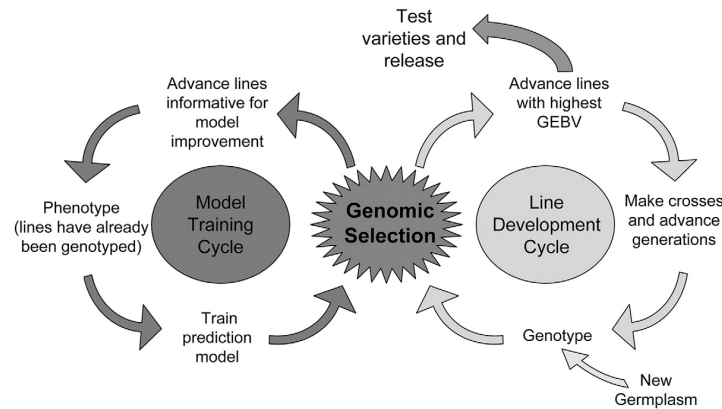


Figure 2. Genomic selection cycle suggested for crop species (Heffner et al. 2011). Breeding cycle time is shortened by removing phenotypic evaluation of lines before selection as parents for the next cycle.

Despite advances in the efficiency of genotyping technologies, genotyping is still costly for forest trees. For example, the Illumina SNP genotyping platform currently costs about \$150 per sample for loblolly pine, though the cost is decreasing. The North Carolina State University Cooperative Tree Improvement Program (www.TreeImprovement.org) has been working on genotyping by sequencing technology, and we expect that the cost of genotyping using this technology could be around \$50 in 2012. The Cooperative is revising its breeding strategies to incorporate these new technologies in coming years. Several GS strategies for *Pinus taeda* breeding populations are being discussed within the program, and a draft GS plan is given in Figure 3 for one of our elite breeding populations with an effective population size $N_e=20$.

In the diagram given in Figure 3, the process starts with creating a training population with an effective population size (N_e) smaller than 50 parents. In this example, 20 Parents are used. Relatedness among 20 founders is desired because that will make the marker-based model more powerful to predict GEBV by tracing historical LD in the population. From full-sib crosses of 20 parents, about 1000 individuals can be genotyped. This progeny population is field tested and breeding values are obtained. Deregressed breeding values of 1000 individuals or phenotypic values adjusted for fixed effects can be obtained to use as new 'phenotype' for development of a marker-based model (M1).

There are different methods to validate the predictive ability of markers. An additional 500 progeny from the same crosses (with known phenotype and genotypes) can be used as a validation population. Alternatively, random sampling of a small subset of progeny or selection of subset of progeny within each full-sib family can be used to validate model M1. This step is a proof of concept to show that the model has predictive power, and is not necessarily an application of GS. In order to utilize the benefit of GS approaches, we need to cross selected individuals from the training population, obtain seeds, and use M1 to make selection decisions. This can be called 'across generation' GS application. The M1 model can be retrained when more genotypic and phenotypic data become available as breeding progresses (M2). GS training models would have more reliability as new data are included and can be used for multiple generations.

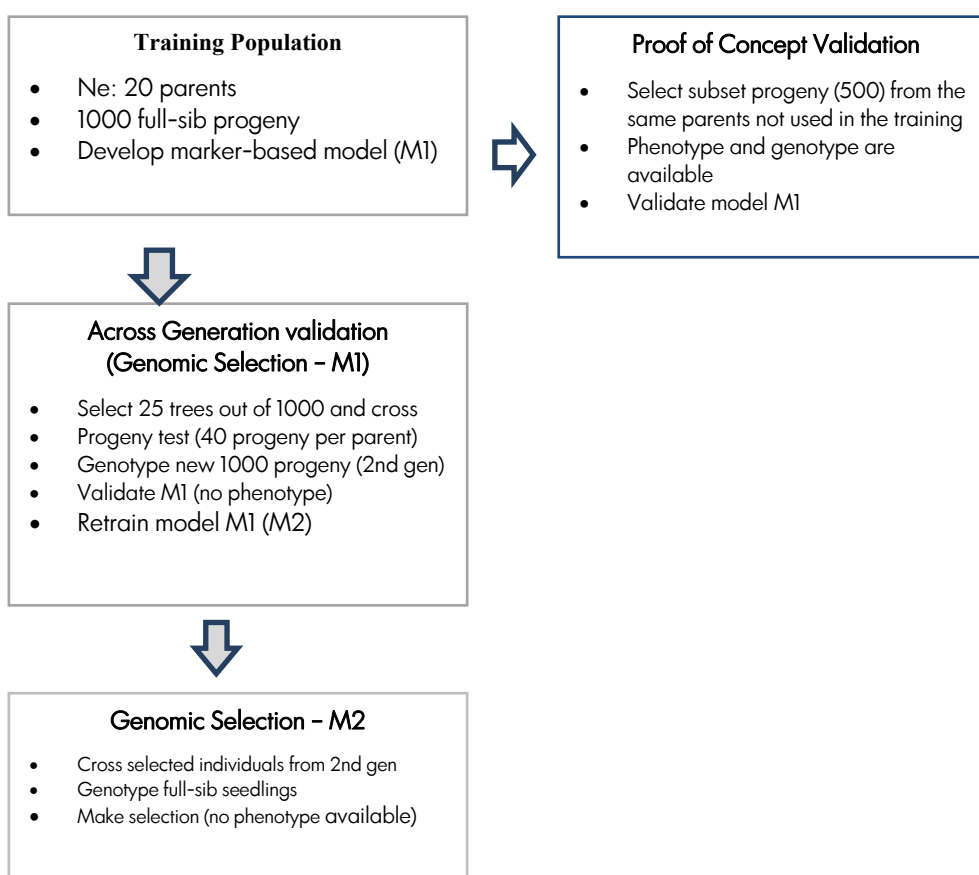


Figure 3. Genomic selection process for an elite breeding population. Marker-based prediction model is retrained across multiple generations. Such process would make the model more powerful for genomic estimated breeding values to trace LD of markers and QTLs.

Key words: Tree breeding, genomic selection, loblolly pine, marker aided selection

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Orchard Seedlot Rating in British Columbia, Canada

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Abstract: Seedlots produced in seed orchards in British Columbia (BC) are routinely assigned numerical values that represent estimates of genetic worth (GW) and genetic diversity, measured by effective population size (N_e). GW is used to predict the increase in wood volume at rotation that results when planting orchard seed as compared to wild seed sources. A minimum N_e for seedlots deployed on public land is intended to ensure that adequate genetic diversity is maintained to deal with unpredictable contingencies in future plantations. GW and N_e are calculated for each seedlot through a seedlot rating system.

Data required for the seedlot rating system include: a) parental breeding value (BV), and b) a measure of female and male gametic proportional contribution. Parental BV is calculated through analysis of progeny test performance. Protocols for collecting the proportional gametic contribution data and their relative merits will be described. The methods used range from basic visual estimates of female and male cone counts to actual seed numbers following extraction. DNA fingerprinting studies have been conducted to test the validity of the various orchard data collection. The discussion extends to the implications for orchard management at one of BC's largest orchard complexes, Kalamalka Seed Orchards, operated by the Tree Improvement Branch of the BC Ministry of Forests, Lands and Natural Resource Operations.

Key words: Seed orchard, seedlot rating, seedlot genetic worth.



POSTER PRESENTATIONS



Spatial Genetic Structure and Mating System of *Pinus densiflora* Seed Orchard Based on Microsatellite Marker Analysis

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Abstract: Genetic structure of clonal seed orchard is one of the main factors affecting mating system. While random arrangement of clones has been generally based on clonal names, establishment of seed orchard based on genotype will be available as the method of DNA profiling is well developed. Anmyeon seed orchard of *Pinus densiflora* established in Korea in 1977 consists of 2,885 ramets of 197 clones producing seeds used in national plantation. However, mating system which affects genetic characteristics of seeds has been rarely studied in this seed orchard. In addition, spatial genetic structure analysis as the preliminary data of mating system research is insufficient. Therefore, the objective of this study was to assess genetic characteristics of Anmyeon seed orchard.

DNA extraction and GPS point data acquisition were conducted from each ramets in the seed orchard. Open-pollinated progenies from 114 ramets were collected to study mating system. Genotypes of each ramets and progenies were analyzed using four microsatellite markers. Four to twenty alleles were appeared in the four markers. The average of expected heterozygosity of ramets and progenies were .699 and .688 respectively. Spatial aggregation index were .993 which was not significantly different from 1 meaning random distribution. Meanwhile, Allelic aggregation index was .776 ($p < .01$) showing non-randomness of spatial genetic structure. It was confirmed that random arrangement of clones based on clonal name cannot assure the random distribution of genotypes. Outcrossing rate was 93.9% which showed similar result with previous study in the same seed orchard. Although further analysis should be done with increased number of markers and combined interpretation of the results, it is regarded that the results of this study will be useful not only in the genetic diagnosis of existing seed orchard, but in the establishment of new seed orchard.

Key words: spatial genetic structure, mating system, seed orchard, microsatellite, *Pinus densiflora*.



Conal Propagation of Superior *Chamaecyparis pisifera* Tree by Cutting in Korea

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Abstract: *Chamaecyparis pisifera* was introduced from Japan in 1920th and planted interior and southern parts (38° 00'–34° 00') of Korea. Timber quality of *C. pisifera* is not good as *Chamaecyparis obtusa* but superior in growth, cold tolerance, air pollution, dry tolerance and shade tolerance. Therefore, superior trees of *C. pisifera* would be a good option to plant as a substance at interior regions instead of *C. obtusa*. This study was conducted to develop cutting propagation method of the mature tree of *Chamaecyparis pisifera* in a short period of time. Cuttings were collected from six superior trees (30 years old) in a *Chamaecyparis pisifera* plantation. We investigated rooting characteristics depending on clone, cutting time (mid March, mid April and Mid June), and auxin treatments (IBA 100ppm mixed with talc powder, Rootone) in a vinyl greenhouse equipped with an automatic fog system without heating. Rooted cuttings were transplanted to a nursery bed, and transplantation survival rates were investigated in order to check whether the cuttings were successful or not for plantation. Cutting time in mid-March was showed better rooting rates with 43.7% compared to April (29.1%) and June (27.6). There was highly significant difference of rooting rate and root number among clones and auxin treatments, but no difference of shoot production. Rootone treatment showed best rooting rate of 80.4% and root number of 18.0, and this results were 2.5 and 3.5 times higher than IBA 100ppm and control. Average survival rate of transplanting rooted cuttings was 97.4% and we didn't see any differences between treatments. It was possible to transplant rooted cuttings until early July. It was concluded that clonal mass propagation of *Chamaecyparis pisifera* by cutting could be possible from the following processes: collect cuttings in March; treat the Rootone; plant the cuttings in a greenhouse

equipped with an automatic fog system; transplant rooted cuttings in early July after hardening; transplant in the next year in the field.

Key words: automatic fog system, mass propagation, mature tree, rooting rate, survival rate.



Variation of Seedling Morphology in Manna Ash (*Fraxinus ornus* L.)

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Abstract: Variations of height and root collar diameter on 1+0 seedlings grown from 28 open pollinated families were examined in Manna Ash (*Fraxinus ornus* L.). Large differences were found both among families and within family for the characters. Averages of seedling height and root collar diameter were 7.05 cm and 2.95 mm, respectively. They varied between 4.50 and 9.63 cm for the height, and between 2.35 and 3.45 mm for the diameter. While about all of the seedlings were unsuitable for the seedling height for plantation, it was 56.4% for the root-collar diameter based on seedling quality classification of Turkish Standard Institute. But, it was 13% for morphologically. Variations among seedlings within clone were 61% for height and 24% for root collar diameter, while they were 0% for among clones. 520 seedlings suitable morphologically were planted to seedling material lot by spacing 2x2 m. Present and future results of the study will make valuable contributions to nursery practice, afforestation, genetic and breeding of the species.

Key words: Seed, Genetic, Breeding, Progeny test, Clone.

Introduction: Turkey has three natural ash species, *Fraxinus excelsior*, *F. ornus* and *F. angustifolia*, which cover 11700 hectares. In these species, *F. ornus* occupy in arid zones of Mediterranean region in Turkey. It also occupies in France, Spain, Italy, Syria and Balkan countries (Saatcioglu, 1976). Wood of the species is an important material for such as furniture, ornamental, charcoal and music industries. Besides, oil is extracted from its bark used in medicinal purposes (Ansin and Ozkan, 2006). In addition to the species is very resistance to aridity, forest fire (Chiatante et al., 2003), and climate change (Tiner et al., 2000). These

characteristics of the species are getting importance of increasing of present occupation by plantation forestry. Seedling is one of the most important factors in success of biological and economical in plantation forestry. The study was carried out to contribute nursery practice, breeding and plantation forestry of the species.

Material and Methods: The seeds were collected at 30 open-pollinated families (Figure 1) sampled phenotypically from a natural population at Egirdir, Isparta located at latitude 37°44' N, longitude 30°49' E, and average elevation 1600 meter in November of 2008.



Figure 1. Mature seeds in Manna Ash.

Collected seeds were stratified at +4°C in humid area by sand during three months. Stratified seeds were sown to Suleyman Demirel University Forest Nursery in 13x25 cm polyethylene bags as three replicates in second week of March, 2009. The seeds germinated in middle of April (Figure 2), while seeds of two families had no germination. Routine nursery practice was applied to the seedlings by beginning of September.



Figure 2. Germination in families.

Height and root-collar diameter data were collected from 599 containerized seedlings which were one year old of 28 families (Figure 3).



Figure 3. Some of measured seedlings.

Seedlings were also classified for quality classification of Turkish Standard Institute (TSE, 1988).

The following linear model was used for the ANOVA in the study:

$$Y_{ij} = \mu + C_i + e_{ij}$$

where Y_{ij} is the observation from the j^{th} seedling of the i^{th} family, μ is overall mean, C_i is the random effect of the i^{th} family, and e_{ij} is error (environment).

Broad-sense heritability (H^2) was estimated as:

$$H^2 = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_e^2}$$

where σ_c^2 is the variance among clones, σ_e^2 is the variance within clone. Variance component, expressed as coefficient of variation among clones (CV_c) in percent, was estimated as: $CV_c = 100\sigma_c / \bar{x}$, where \bar{x} is overall character mean.

Results and Discussion

Averages of height and root collar diameter, and their relations: Total averages of height and root collar diameter were 7.05 cm and 2.95 mm, respectively (Table 1). The averages were reported 38.6 cm and 2.90 mm for Turkish manna ash by Yucedag and Gezer (2007), respectively. The difference of seedling height could be from seed population, nursery location number of samples seed trees. It was also emphasized by i.e. Urgenc (1992), Demirci and Bilir (2001).

Table 1. Averages, ranges and standard deviations for height and root collar diameter.

	Average	Minimum	Maximum	Standard deviation
Height (cm)	7.05	1.20* - 4.5**	21.0* - 9.63**	2.77
RCD (mm)	2.95	1.30* - 2.35**	5.70* - 3.45**	0.75

*, seedling level, **, family level.

There were large differences among families and within family for studied characteristics (Table 1). For instance, the height was varied between 9.63 and 4.50 cm in families, while it was between 1.20 and 21.0 cm in seedlings. The large differences were also found among seedlings of the same family (Figure 4). The seedling differences were four times (1.30 mm and 5.70 mm) for root collar diameter as given in Table 1. The result was also well accordance with result of analysis of variance. The variations were larger in height than root collar diameter among families according to results of Duncan's multiple range test.



Figure 4. Growth differences among seedlings of the same clone.

The large differences among families and within family showed the large genetic diversity in the species although its restricted sampled area and natural occupation of the species. Large differences were reported by Yucedag and Cengiz (2007) for Turkish Manna Ash. Large differences were also emphasized in Fraxigen (2005) report.

Positive and significantly correlation ($r=0.719$, $p<0.05$) was found between seedling height and root collar diameter (Figure 5).

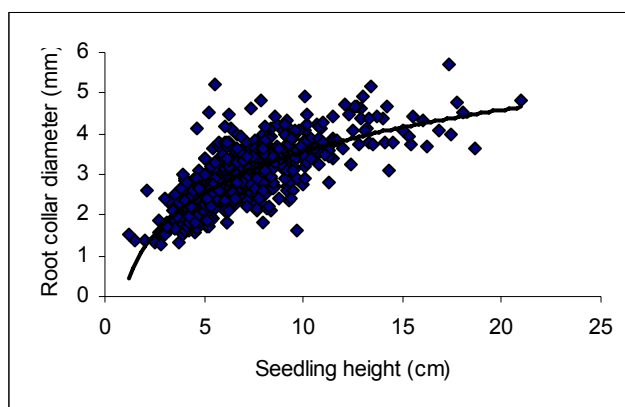


Figure 5. Relation between seedling height and root collar diameter.

Seedling Quality: Only 0.2% of seedlings were suitable for plantation according to seedling quality classification of Turkish Standard Institute for height, while it was 43.6% for root collar diameter (Table 2). 87% of seedlings were suitable morphologically (color, biotic damages etc.)

Table 2. Quality classification and distribution of seedlings.

Height (H)		RCD		H+RCD	
Quality (≥ 20 cm)	Cull (<20 cm)	Quality (≥ 3 mm)	Cull (<3 mm)	Quality ($H \geq 20$ cm, RCD ≥ 3 mm)	Cull ($H < 20$ cm, RCD < 3 mm)
0.20 %	99.8 %	43.6 %	56.4 %	0.20 %	99.8 %

Results of the present study showed that quality classes of Turkish Standard Institute were not applicable for the species. Similar results were also reported by Yucedag and Gezer (2007), and Gezer and Yucedag (2006). The same quality classes of Turkish Standard Institute were given for all *Fraxinus*, *Acer*, *Sophora*, and *Ulmus* species. However, Cicek et al. (2007) reported 58 cm height and 10 mm root collar diameter *Fraxinus angustifolia*. These large differences among ash species were also showed the inapplicable of the quality classes.

Heritability: Variations among seedlings within clone were 61% for height and 24% for root collar diameter, while they were 0% for among clones. Mother trees were sampled from a narrow area. So, the differences could be parental effect. However, the results could be changed for populations and characters.

Conclusions: Seedling quality classes should be carried out in the species alternatively of Turkish Standard Institute.

The seeds were sampled at a restricted area and family numbers. It could be larger in future studies.

The results of nursery stage could be combined by field performance of the seedlings. Seedlings suitable morphologically were planted to seedling material lot by spacing 2x2 m (Figure 6).



Figure 6. Plantation area of the seedlings, and two years plantation.

The results of field stage will be presented near the future.

Acknowledgement

I thank to "The Scientific and Technological Research Council of Turkey-TUBITAK" for financial support (Project No: TOVAG-1080611).

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Genetic Characteristics of a Seed Orchard (*Pinus silvestris* L.) in the Susz Forest District (53° 43'12" N, 19° 20'14" E)

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Abstract: The aim of this research was to describe the genetic variety of clones coming from the mother trees growing in the north eastern Poland. The described clones are located in a seed orchard in the Susz forest district (53 ° 43'12 "N 19 ° 20'14" E). Laboratory tests were carried on 222 plants, including a parent tree and clones derived from it. The tests were based on the analysis of isoenzymatic proteins within which a genetic variation of 13 isoenzymatic systems was identified: *Est-B*, *GDH*, *AdhA*, *AdhB*, *SdhA* *SDHB*, *PgdB*, *MdhA*, *MdhC*, *Goth*, *Goth B*, *GotC*, *DiaC*. The chemical composition of buffers used in the experiment was based on the commercial patent "iso 13", patented by I. Odrzykoski at Adam Mickiewicz University. The acquired results allow to describe the basic genetic characteristics of the population and to determine the overall percentage of errors in the seed orchard.

The expected number of alleles per locus in the population is 2.9, while the effective number of alleles is 1.5. The observed heterozygosity is 0.32, while the expected heterozygosity is 0.30. Wright fixation index - 0.03. The Genetic structure of the population is in Hardy Weinberg equilibrium. The number of heterozygotes is larger than expected, the ratio $F = -0.03$.

The research showed that there are significant statistical differences ($P = 0.01$) between the frequency in the received alleles in *SdhA* gene. 5 alleles were identified in the gene. Their frequency was: allele 1 (88%), 2 (9%), 3,4,5 (1%).

A molecular analysis was carried out which allowed to describe the overall error rate on the seed orchard. What is meant by a *general error* is growing a different crop than expected in a particular place of the seed orchard. The percentage of

error plants in the tested population was 41%. They were removed from further research.

In conclusion there were more heterozygotes than homozygotes in the studied population. In the specific traits for breeding oriented plantations, the observed genetic variation is positive and, besides selection, provides the ability to adapt to changing climate conditions of the studied population.

Attention should be paid to the described errors that significantly disrupt the breeding selection process.

Key words: genetic variability, isozyme markers, *Pinus silvestris*.



Breeding without Breeding Strategy and its Application (not only) in the Czech Republic

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Introduction: Tree improvement program of Scots pine (*Pinus sylvestris* L.) in the Czech Republic can be classified as low-input breeding, featuring large number of first generation seed orchards (large number of clones) and either open-pollinated, or unstructured progeny trials.

Contemporary DNA fingerprinting methods enable to convert extant open-pollinated families or unstructured plots to a series of full-sibs nested within half-sib families. This approach is called Breeding without Breeding (BWB) can considerably accelerate breeding (El-Kassaby and Lstiburek 2009).

Key words: Breeding without Breeding, microsatellites, Scots pine.

Material and methods: Scots pine is with its proportional representation of 17% the second most important forest tree species in the Czech Republic. However, generally poor genetic quality of present pine forests (e.g. large variability in stem form) requires further application of breeding activities.

This project started as a pilot activity in two existing seed orchards and half-sib progeny tests. Next, 600 superior individuals from each half-sib progeny test (app. 10%) were selected based on their height (ht), diameter at breast height (dbh), straightness, and branching. This was the first stage of selection prior to genotyping (El-Kassaby and Lindgren 2007).

All parents in the first-generation seed orchards along with the top-ranking 600 progenies were genotyped. Dormant vegetative buds were collected and immediately stored at -80°C to avoid DNA degradation. DNA was extracted using Invisorb® SpinPlant Kit. The selected trees were genotyped for ten microsatellite DNA markers (SSRs), SPAC 11.4, SPAC 11.6, SPAC 12.5 (Soranzo et al. 1998), LOP 1 (Liewlaksaneeyanawin et al. 2004), PtTX 2146, PtTX 3025, PtTX 3107,

PtTX 4001, PtTX 4011 (Aucland et al. 2002) and SsrPt_ctg 64 (Chagné et al. 2004).

Results and conclusion: Our results proved high polymorphism level (PIC) of selected markers (Table 1).

Locus	k*	HObs	HExp	PIC
SPAC 11.4	17	0.640	0.804	0.787
SPAC 11.6	46	0.418	0.954	0.952
SPAC 12.5	48	0.847	0.940	0.936
LOP 1	14	0.445	0.661	0.616
PtTX 2146	21	0.757	0.773	0.744
PtTX 3025	12	0.508	0.534	0.478
PtTX 3107	11	0.272	0.809	0.784
PtTX 4001	14	0.770	0.740	0.711
PtTX 4011	12	0.508	0.695	0.649
SsrPt_ctg 64	14	0.331	0.371	0.349

Allele frequency analysis (**k** – Number of alleles at the locus, **HObs** – Observed heterozygosity, **HExp** – Expected heterozygosity, **PIC** – Polymorphic information content)

The evaluation of parent trees and their progeny (pedigree reconstruction) was done using software Cervus (Kalinowski et al. 2007). The full-sib structure was revealed from sib-ship analysis using the program COLONY (Wang 2004). Individuals for the second-generation seed orchard (second stage of the selection) will be chosen according to their breeding values and the desired effective population size.

Two second-generation seed orchards will be established in 2013-2014. Following this successful project, BWB is considered the major tree breeding strategy for Scots pine and Norway spruce in the Czech Republic.

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Selection for improvement of resistance of Scots pine against needle cast

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Abstract: Results from experiments containing altogether 30 provenances represented by 489 open pollinated families reveal that provenance region within Latvia has not significant influence on differences in resistance against needle cast, but provenance and family has. Progenies of first round (phenotypically selected) seed orchards were not more resistant than stand progenies, indicating that selection for height and quality had not resulted in improvement of resistance. Ranking of families and provenances based on needle cast damage level was stable across sites ($r=0.74$ on average), and resistance was strongly linked to survival of trees ($r=-0.69$ and $r=-0.42$ at provenance and family mean level respectively).

Selective collection of seeds from Smiltene clones in seed orchards could serve as immediate source of material with higher resistance for pine regeneration in areas severely affected by disease.

Key words: *Lophodermium seditiosum*, tree breeding, adaptation

Introduction: Needle cast (*Lophodermium seditiosum* Minter, Staley & Millar) is pathogenic fungi species that affect one-year old needles of Scots pine. It is predicted to increase in importance due to management trends (fewer, smaller seedlings planted) and changes in climatic conditions (milder autumns, winters).

Study was carried out to assess the occurrence of the disease and possible ways of selection for more resistant material for propagation.

Materials and methods: Data for the study was obtained from 3 trial series:

a) set of 2 nursery trials, located in western and eastern part of country. Altogether 282 open-pollinated families from 15 provenances located across Latvia were

included in one of the trials and fraction of the material in another. 8 trees per replication were planted; 3,3 replications in average per family in one site and 1 replication in other. Needle cast assessment carried out in 2nd growing season;

b) nursery trial in eastern part of Latvia, including 7 provenances, represented by altogether 207 open-pollinated families. Needle cast damages were assessed in 2nd growing season on 10 randomly selected trees per family;

c) geographical provenance trial, including altogether 56 provenances from Russia (3), Poland (7), Eastern part of Germany (27) and Latvia (18) as well as average samples from six first round (phenotypically selected) Latvia's Scots pine seed orchards. Material was planted in 3 geographically and climatically different sites in Latvia with similar site conditions. Needle cast were assessed in 8th growing season for all trees (35 tree block plots in 6 replications per site).

The level of needle cast damage was classified in five grade scale, where Grade 1 corresponds to 0-5% of needles damaged by disease in the shoots of last year; Grade 2 – 6-35%; Grade 3 – 36-65%; Grade 4 – 66-95%; and Grade 5 – 96-100% of needles damaged by needle cast.

Site (trial location) was significant factor determining the damage grade – it ranged from 3,4 to 4,5 on average.

Results reveal, that disease was more pronounced in sites with milder climatic conditions Liepaja and Zvirgzde (western part of Latvia) than in Kalsnava (eastern part of Latvia). Comparison of temperature climate (last 30 years) in the end of summer - beginning of autumn using data from closest meteorological stations reveal, that differences between the eastern and western sites were 2.2°C on average.

Scots pine provenances from different part of Latvia (provenance regions) did not contrasted significantly in the level of needle cast damages. Analysis of provenances separated by larger geographical distances reveals statistically significant but practically negligible differences (Fig. 1.).

Provenance as well as family was a significant factor determining the needle cast damage grade in all trials.

Strong ($r=0.71-0.77$) and significant provenance mean correlation between test sites for needle cast damage grade was found, indicating relative stable performance of families an low number of test sites needed to determine the most resistant material.

Needle cast had strong and significant effect on survival in both sets of nursery trials. Correlation between needle cast damage grade and survival in nursery trial

was strong and significant both at provenance and family mean level ($r=-0.69$ and $r=-0.42$ respectively, $p=0.001$).

Analysis does not reveal significant differences in initial height of families with mean needle cast grade 3 and 5, but differences in height increment were significant. That indicates the hampering effect of the disease on growth of least resistant families.

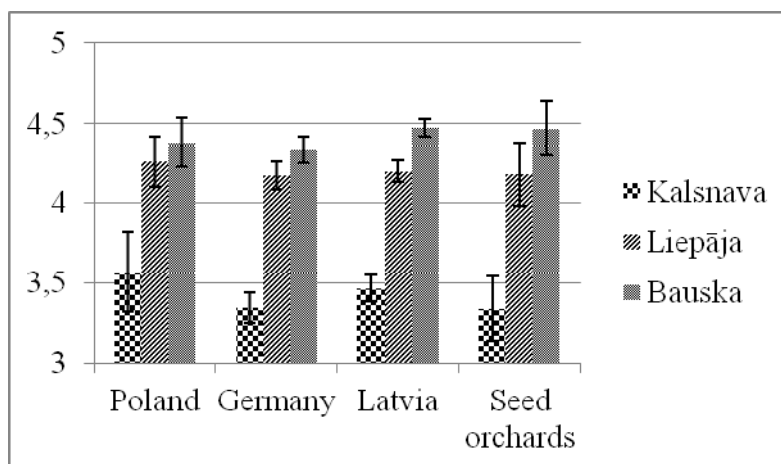


Fig.1. Average needle cast damage grade for different Scots pine provenances from 3 geographically distant regions and average samples from Latvia's seed orchards planted in 3 test sites.

In one of the nursery trials proportion of tree with most serious needle cast damages (grade 5) was found to be highest in provenances Smiltene and Ūķene. For those populations influence of needle cast grade on height increment was non-significant (Table. 1.) in contrast from other provenances. This finding suggests different at provenance level not only in susceptibility of disease, but also in effect of its damages on tree growth and survival.

Phenotypic selection of clones based on height growth and quality does not necessarily yields improvement of resistance against needle cast. However, collection of seed material from clones of most resistant provenances that are represented in seed orchards could lead to immediate improvement of resistance of plant material and could be used to provide solutions (plants) for most affected areas. Resistance tests needs to be carried out for selection of least affected families with breeding program for selection of new sets of clones for seed orchards as long-term solution.

Table. 1. Average height increment and survival of trees from different populations in connection with level of needle cast damages in second growing season.

Provenece	Trait	Needlecast damage grade				Significance of needle cast damage
		2	3	4	5	
Ūķene	height increment, cm	22,5	21,0	20,1	18,0	$\alpha > 0,05$
	survival, %	83	79	75	73	
Smiltene	height increment, cm	24,8	25,5	22,3	22,2	$\alpha > 0,05$
	survival, %	87	86	84	84	
Tukums	height increment, cm	-	27,3	22,5	18,5	$\alpha > 0,01$
	survival, %	-	88	80	65	
Misa	height increment, cm	26,5	23,1	19,8	17,8	$\alpha > 0,01$
	survival, %	90	79	72	56	



Variation in Clone Fertility and Genetic Diversity of Seed Crop from an *Abies alba* Mill. Seed Orchard

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Abstract: Silver Fir (*Abies alba* Mill.) is one important - economical and ecological - species from Romania. Commonly, it is naturally regenerated, but in the recent years, the windfall damages and the interest for mixed stands increased its interest for reforestation, based on seeds from seed orchards. In the frame of Romanian breeding program, eleven clonal seed orchards were establish during 1976-1982. The present study was carried out in a first generation Silver fir seed orchard (Gârcina, northern Romania), in order to investigate within- and among clone variation in reproductive energy (cone production) and their effect on the genetic diversity of seed crop. Based on genetic markers, the genetic diversity of the parental and progeny generations was compared. The frequency of flowering trees was about 74% in 2010 and a broad variation was found in cone production between clone and ramets. Implication of this variation in genetic composition of orchard seed production, seed crop management decision and advanced seed orchard establishment are also presented.

Key words: *Abies alba*, genetic diversity, cone production.



“Neglected Areas” As A Small Urban Seed Orchard

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Abstract: Today, vacant and abandoned residential gardens are often left to its own devices is undergoing a very invasive plant species invasion. Frequently observed in Turkey as in America and Europe, this situation leads to the spread of the environment of these areas a lot of seed. On the one hand is engaged to dispose of unwanted plants garden enthusiasts, on the other hand, leaving the natural plant species which aims to educate its own devices elicit different opinions environmental approaches. This article neglected to serve the remainder of these areas and biological diversity in the city, although small due to the current biotopes emphasizes the importance of the seed orchards.

The purpose of this article or forgotten in the city abandoned by human beings in space and in plots with trees, shrubs and wild plants in the landscape contrary to the appearance of evil by fulfilling an important function in the urban ecosystem, constituted the smallest seed orchards instead of highlighting a very important duty in terms of landscape ecology brought to reveal. Biological diversity of biotopes from place to place with the City of urbanization continues paused a perfect ecosystem, but the biological clock. Urban life in the natural vegetation, which provides for non-compliance, seasonal color change, the best-known activities such as opening and flower next to fruition the original goal of reproductive and environmental ecosystems is that for the continuity of service required to fulfill the vital functions. One seed in the wind for miles transported, by making the soil ready for planting by the release of a bird, an attempt to grab a pole distances, it can not replace in a tree, shrub, or the reproductive ability of wild plant propagation and biological energy represents not give up. For this reason, many birds, insects, bees and butterflies haunt the city center, albeit a very small and neglected in the empty spaces by looking at only visually perceived as garbage areas, these areas of biological and genetic activities are conducted is very important open air labs. As a

result, empty and abandoned areas of biological importance that hosts a lot of information to investigate the protection and care should be taken as pilot areas.

Key words: Neglected areas, Small Urban seed orchards, landscape ecology, Abandoned residential gardens.