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Allozyme Variation in Eight Natural Populations of *Pinus roxburghii* SARG. in India

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Abstract

Seeds collected from eight populations of Chir pine (*Pinus roxburghii* SARG.) from the natural distribution range of the species in Himachal Himalayas in India were analysed isozymatically at 11 enzyme systems. For the enzyme systems studied, 25 gene loci were identified out of which 18 were polymorphic. The observed mean values for genetic variation were slightly lower than mean values reported for *Pinus* species (number of alleles: 1.65 compared to 2.36; effective number of alleles: 1.13 compared to 1.26; observed heterozygosity: 0.153 compared to 0.179). A small differentiation among populations and large variation within populations were reflected by small value of GST (0.04). Considering the different genetic parameters three populations seem favourable for gene conservation measures.

Key words: *Pinus roxburghii*; allozymes, differentiation, multilocus diversity, genetic distance, variation.

Introduction

Owing to its economical and ecological importance, *Pinus roxburghii* has outnumbered all other species in afforestation programmes in its natural zone of occurrence. Selecting superior *P. roxburghii* stands/genotypes and their mass multiplication can increase its productivity many fold as significant variation could be expected on the basis of its natural distribution under diverse environmental conditions which include heterogeneous areas of the Shiwaliks and western Himalayas. Hence, there is an immediate need to conserve and manage genetic resources of this species. However, establishing priorities for gene conservation (*in situ* and *ex situ*), management and use of tree genetic resources for breeding programmes as well as efficient and successful plantation require an understanding of the degree of diversity within and between populations of a species as geographically separated populations have different genes and their frequencies due to mutation, different selective forces and genetic drift. Within each tree species the amount and pattern of genetic variation determine its adaptability and are consequently essential parameters of the long term stability of the forest ecosystem.

The scientific methods used to distinguish levels of variation are the working tools for shaping the decisions on management and tree improvement policies. Studies conducted in the past on provenance testing of *P. roxburghii* have revealed significant variation in growth parameters, and biochemical contents (QURESHI and SINGH, 1967; SINGH *et al.*, 1970; SAGWAL, 1978; GHOSH *et al.*, 1982; SHARMA, 1986). However, provenance trials, based on quantity measurements often give an incomplete picture about the population structure as these measurements are subjected to environmental influences (BROWN and MORAN, 1978). There is an urgent need to supplement the selection procedure by the modern techniques for detecting systematic differences between populations of this species. The method which has been proved suitable for characterisation and demarcation of subspecific groupings of plant/animal species is the analysis of isoenzymes.

Since there is no report based on isoenzyme data in *P. roxburghii* in India, aim of the present study is to assess the genetic variation at isoenzyme level present within and between its populations growing under different environmental conditions and hence to describe the genetic architecture of the species (SHARMA, 1999). HUSSAIN (1995) studied twelve populations of *P. roxburghii* of the species' natural distribution westward of India.

Material and Methods

Seeds collected from eight natural populations of *P. roxburghii* viz., Sulyali (P1), Aghar (P2), Banethi (P3), Gagret (P4), Nauni (P5), Nihari (P6), Shilly (P7) and Shalaghat (P8) covering Shiwalik and Himalayan ranges of species' distribution in Himachal Pradesh in India (*Figure 1*) were used for these studies. The distance between the populations varied from 4 to 233 km.

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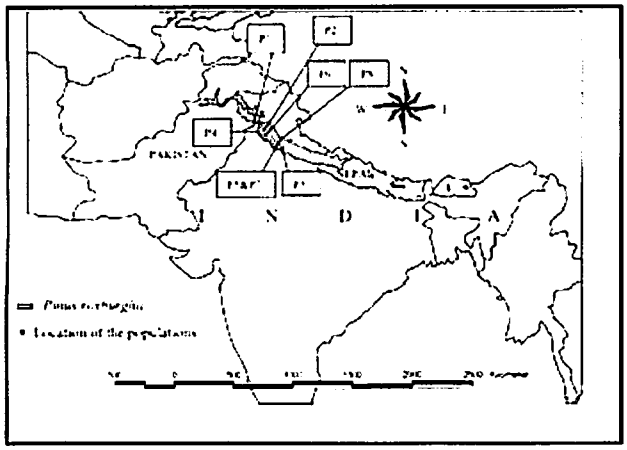

Code No.	Population	District	Altitude (m)	Latitude (°N)	Longitude (°E)
P1	Sulyali	Kangra	490	32.3	75.9
P2	Aghar	Hamirpur	800	31.7	76.5
P3	Banethi	Nahan	1370	30.6	77.3
P4	Gagret	Una	535	31.5	76.3
P5	Nauni	Solan	1450	31.3	76.4
P6	Nihari	Bilaspur	1200	30.8	77.1
P7	Shilly	Solan	1550	30.9	77.1
P8	Shalaghat	Shimla	2000	31.1	77.2

Figure 1. – Map showing distribution of *Pinus roxburghii* and locations of the populations.

From each population twenty trees of different ages (thirty to more than hundred years) were selected keeping minimum distance of 50 m between tree to tree. Eight embryos per mother tree were analysed to get the information on 160 diploid samples per population. The data generated using eight embryos per mother tree were used to calculate the genetic parameters. In total, 2560 seed samples (embryos and endosperms) were analysed for 11 enzyme systems viz., Aconitase (ACO), Aspertate aminotransferase (AAT), Glutamate dehydrogenase (GDH), Isocitrate dehydrogenase (IDH), Leucine-amino peptidase (LAP), Malate dehydrogenase (MDH), Menadione reductase (MNR), Phosphoglucose isomerase (PGI), Phosphoglucomutase (PGM), 6-Phosphogluconate dehydrogenase (6PGDH) and Shikimic acid dehdrogenase (SKDH) using horizontal starch gel electrophoresis based on the methods given by SHAW and PRASAD (1970), CONKLE et al., (1982), and CHELIAK and PITEL (1984). For details, see SHARMA and VON WUEHLISCH (1998).

Data analysis

The data generated were analysed with the help of different computer software programmes like: GSED (GILLET, 1994); Biosys-1 (SWOFFORD and SELANDER, 1981), Popgene (YEH *et al.*, 1997) and GDA & NT (DEGEN, 1998).

The following measures of variation and genetic differentiation were calculated using above programmes. The percentage of polymorphic loci (99% criterion), actual and effective number of alleles/diversity (GREGORIUS, 1987), observed heterozygosity (GREGORIUS *et al.*, 1986), expected heterozygosity (NEI and ROYCHOUDHURY, 1974), Fixation index (WRIGHT, 1965) and Genetic distance (NEI, 1972; GREGORIUS, 1974).

The statistical significance of the calculated values was checked with permutation tests. Moreover, the test of null hypothesis that the observed frequencies of alleles and genotypes in the different populations were random samples from a single population, χ^2 and G-tests (WOOLF, 1957) have been used. To visualise genetic relationships between populations, matrices of genetic distance values were clustered by the unweighted pair-group method using arithmetic means (UPGMA; SNEATH and SOKAL, 1973). In order to apportion genetic diversity to various levels of a hierarchical population, WRIGHT's (1965) set of F-coefficients (FIT, FIS and FST) has been employed.

Results

Number of alleles

The presence or absence of different allelic types gives an estimate of the total genetic variation of a species in any given population. In total, 50 alleles were recorded for 25 gene loci. Gene loci namely: AAT-B, AAT-C, MDH-B, MDH-C, PGM-A, 6PGDH-A and SKDH-A were variable with three alleles each whereas gene loci: ACO-A, GDH-A, IDH-B, LAP-A, LAP-B, MDH-A, MNR-A, MNR-B, PGI-A, PGI-B and PGM-B were variable with two alleles each. Other gene loci (AAT-A, AAT-D, IDH-A, 6PGDH-B, SKDH-B, MNR-C and MDH-D) showed only one allele each.

The bands for the different isozymes moved towards the anode, only AAT-D showed enzyme movement towards the cathode.

Genetic diversity and heterozygosity

The data pertaining to per cent polymorphic loci (P), average number of alleles per locus (A/L, 99% criterion), mean effective number of alleles (v), observed heterozygosity (Ho), expected heterozygosity (He) and fixation index (F) are given in *Table 1*. Based on 99% criterion, the mean per cent polymorphic loci for the population studied was 57.6. The populations Aghar and Nihari revealed the minimum (44.44) and the maximum (66.67) percentage polymorphic loci, respectively. Population Shalaghat showed the maximum average number of alleles per locus (1.75) followed by population Nihari (1.71). The minimum number of alleles were, however, recorded in populations Shilly and Nauni each showing a value of 1.58. The mean value over all the populations was 1.65. The allelic diversity of the gene pool was 1.13. The population Shalaghat reflected minimum allelic diversity (1.087) whereas the maximum (1.156) was

Table 1. – Genetic Variation and Diversity in eight population of *P. roxburghii:* Per cent polymorphic loci based on 99% criterion (P), Average number of alleles per locus (A/L), Mean effective number of alleles (v), Heterozygosity observed (Ho), Heterozygosity expected (He) and Fixation index (F).

Population	Genetic diversity					
⇓	P	A/L	v	Ho	He	F
SULYALI	61.11	1.67	1.139	0.160	0.162	-0.020 n.s.
AGHAR	44.44	1.63	1.135	0.161	0.159	-0.017 n.s.
BANETHI	55.56	1.63	1.152	0.169	0.176	0.034 n.s.
GAGRET	61.11	1.63	1.118	0.134	0.141	0.051 n.s
NAUNI	61.11	1.58	1.117	0.139	0.140	0.018 n.s.
NIHARI	50.00	1.71	1.137	0.150	0.161	0.059*
SHILLY	66.67	1.58	1.156	0.168	0.180	0.062*
SHALAGHAT	61.11	1.75	1.087	0.096	0.106	0.066*
Mean	**57.6**	**1.65**	**1.130**	**0.147**	**0.153**	**0.039/0.037**

* significant at 5%

Table 2. – Fixation index of eight polymorphic loci

Gene loci	Population							
	SULYALI	AGHAR	BANETHI	GAGRET	NAUNI	NIHARI	SHILLY	SHALAGHAT
ACO-A	-0.053 n.s.	-0.003 n.s.	0.119 n.s.	-0.016 n.s.	0.118 n.s.	0.175*	0.110 n.s.	0.402***
MDH-B	0.081 n.s.	-0.026 n.s.	-0.012 n.s.	0.123 n.s.	0.163*	-0.013 n.s.	-0.005 n.s.	-0.019 n.s.
MDH-C	0.299***	-0.100 n.s.	-0.093 n.s.	0.092 n.s.	-0.006 n.s.	0.183**	0.141*	-0.008 n.s.
MNR-B	-0.283***	0.040 n.s.	0.137*	0.070 n.s.	-0.008 n.s.	0.145*	-0.006 n.s.	-0.025 n.s.
PGI-B	-0.100 n.s.	-0.001 n.s.	-0.067 n.s.	0.023 n.s.	-0.114 n.s.	-0.049 n.s.	0.019 n.s.	0.118 n.s.
PGM-B	-0.042 n.s.	-0.005 n.s.	0.121 n.s.	0.153 n.s.	-0.060 n.s.	-0.066 n.s.	-0.120	-0.006 n.s.
6PGDH-A	-0.026 n.s.	0.044 n.s.	-0.063 n.s.	0.066 n.s.	0.058 n.s.	0.158*	0.230**	0.119*
SKDH-A	-0.039 n.s.	-0.052 n.s.	0.125 n.s.	-0.104 n.s.	0.104 n.s.	-0.059 n.s.	0.129 n.s.	-0.054 n.s.
Mean	**-0.020 n.s.**	**-0.017 n.s.**	**0.034 n.s.**	**0.051 n.s.**	**0.018 n.s.**	**0.059***	**0.062***	**0.066***

n. s. non significant * significant at 5% ** significant at 1% *** significant at 0.1%

revealed by the population Shilly. Interestingly, the population Shilly produced the minimum average number of alleles per locus but showed the highest allelic diversity in contrary to the population Shalaghat which produced the maximum average number of alleles but revealed the minimum allelic diversity.

The mean observed heterozygosity for the populations studied, ranged between 0.096 and 0.169 with a mean value of 0.147. The population Banethi produced the maximum number of heterozygotes about 70% more over the population Shalaghat which gave the minimum number of heterozygotes. The population Banethi was closely followed by the population Shilly (0.168). The expected heterozygosity varied from 0.106 to 0.18 and averaged at 0.153.

The mean values of the fixation index for two of the eight populations studied were slightly negative (*Table 1*). Out of the remaining six populations three namely; Nihari (0.059*), Shilly (0.062*) and Shalaghat (0.066*) showed positive and significant values of fixation index indicating excess of homozygotes. Single locus fixation index values are significant for only few populations (*Table 2*).

Genetic differentiation

Significant differences were observed among the allelic frequencies of the eight populations. Both G-test and χ^2-test were significant for all the polymorphic loci except for GDH-A where the values of both the tests were statistically non significant. Similar to that of allelic frequency distributions, the values of G-test and χ^2-test for genotypic frequency distributions among the eight populations were also significant for all the gene loci except GDH-A.

Genetic distance

The matrix pairwise genetic distance DG (GREGORIUS, 1974) and DN (NEI, 1972) calculated for eight *P. roxburghii* populations is given in *Table 3*. Genetic distance values DG ranged from 0.023 between populations Gagret and Nihari to 0.063 between populations Aghar and Shalaghat and averaged at 0.043. Out of the 28 pairwise combinations, 16 i.e. about 57% showed values of the genetic distance more than 0.040 whereas about 43% gave values less than 0.040. Population Shalaghat always showed higher genetic distances, more than 0.040 with all other populations.

The average values of gene pool genetic distance calculated using all 24 gene loci were small. Like that of GREGORIUS genetic distance, the minimum and the maximum values of the NEI's genetic distance were also observed between populations Gagret and Nihari, and Aghar and Shalaghat, respectively (*Table 3*). The mean NEI's genetic distance was 0.006, showing that the differentiation among the populations is low. Though the genetic distances between the populations were small yet the values were highly significant (at 0.01%) for both the measures of genetic distance (GREGORIUS and NEI).

Table 3. – Gene pool genetic distance (GREGORIUS, 1974; above diagonal and NEI, 1972; below diagonal) among eight populations of *P. roxburghii*

Population ↓ ⇒	P1	P2	P3	P4	P5	P6	7	8
SULYALI		0.044***	0.047***	0.036***	0.046***	0.038***	0.051***	0.052***
AGHAR	0.006***		0.045***	0.035***	0.043***	0.045***	0.048***	0.063***
BANETHI	0.009***	0.008***		0.034***	0.037***	0.035***	0.045***	0.051***
GAGRET	0.004***	0.005***	0.005***		0.031***	0.023***	0.036***	0.042***
NAUNI	0.010***	0.008***	0.005***	0.003***		0.035***	0.038***	0.050***
NIHARI	0.004***	0.006***	0.003***	0.002***	0.004***		0.037***	0.049***
SHILLY	0.007***	0.006***	0.006***	0.004***	0.004***	0.004***		0.061***
SHALAGHAT	0.008***	0.012***	0.009***	0.006***	0.011***	0.007***	0.013***	

*** significant at 0.1%

The dendrogramme obtained with the unweighted pair-group method using arithmetic means (UPGMA) for GREGORIUS genetic distance is shown in *Figure 2*. As evident from the dendrogramme, clustering resulted in formation of only single group of the populations studied. The populations Gagret and Nihari showing the minimum genetic distance were the first to form the group. There was no clear grouping of the populations according to geographic pattern, however, the populations Sulyali and Shalaghat from two extreme altitudes were found to be most loosely connected with other populations.

F-statistics

Data on the correlation between uniting gametes within populations (FIS), among populations (FST) and for the eight studied populations (FIT) were compiled. The FIS values varied from –0.037 (AAT-C and PGM-B) to 0.183 (ACO-A) and averaged at 0.040. The negative values at gene loci AAT-B (–0.011), AAT-C (–0.037), GDH-A (–0.006), MDH-A (–0.013), PGI-B (–0.022) and PGM-B (–0.037) indicated an excess of heterozygotes within the populations. Whereas positive values of FIS at other gene loci reflected an excess of homozygotes. The mean positive value (0.040) as a whole exhibited an excess of homozygotes within the populations. At the same time, FIT

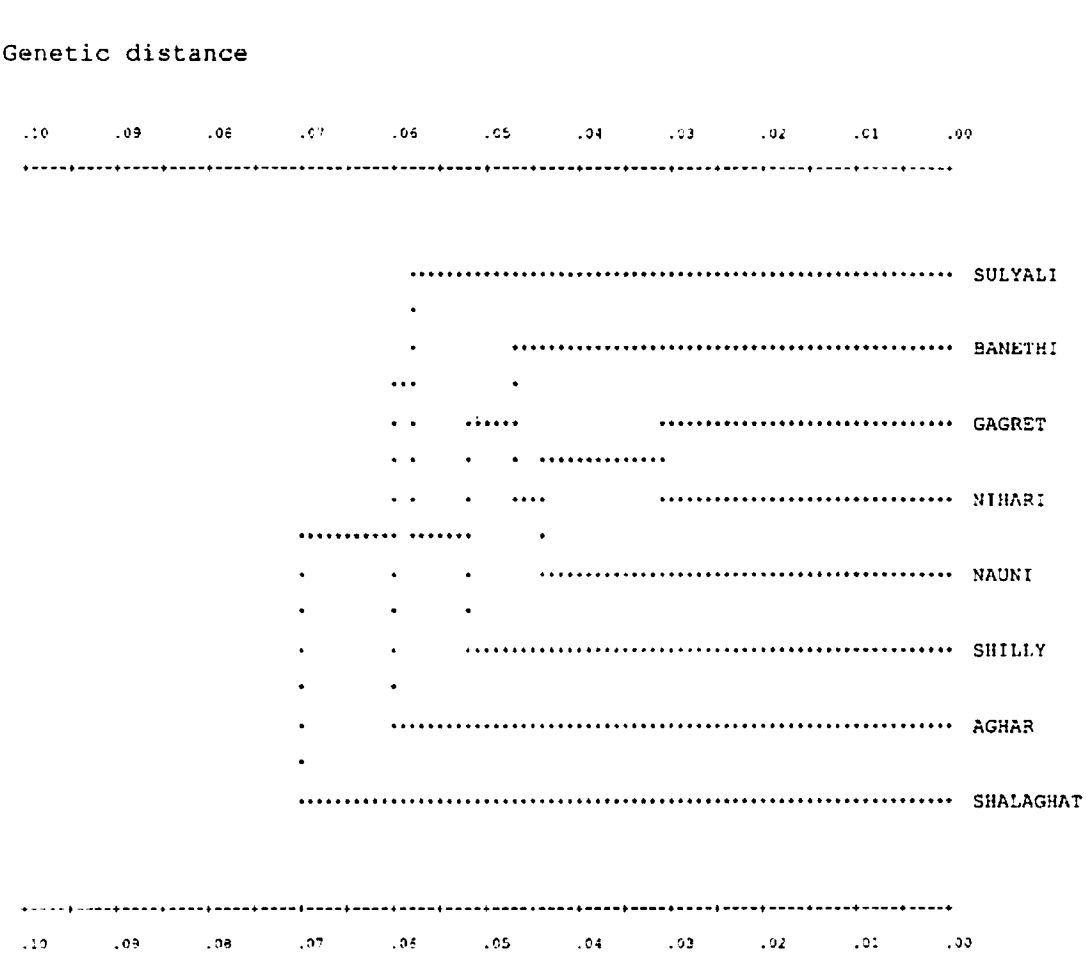

Figure 2. – Dendrogramme of eight populations of *P. roxburghii* based on genetic distance.

values varied from –0.014 (PGI-B) to 0.255 (ACO-A) with a mean value of 0.079 indicating heterozygote deficiency in the studied populations of *P. roxburghii*. FST which measures the level of interpopulation diversity varied among the loci, ranging from 0.006 (AAT-B and GDH-A) to 0.117 (IDH-B) and averaged at 0.041. It means about 96 % of the genetic variation in *P. roxburghii* resided within the populations and only 4 % among the populations.

Discussion

Isozyme gene loci

In this study 11 isozyme systems were studied, which is the same number as studied by HUSSAIN (1995). However, the allozymes studied by HUSSAIN differ slightly from the ones analysed in the present study. HUSSAIN identified five loci coding for MDH which are all polymorphic with two or more alleles each, whereas we found only four loci, of which MDH-D was monomorphic (SHARMA and VON WUEHLISCH, 1998). HUSSAIN investigated NADH which we did not, instead, we studied PGI-A and PGI-B, which HUSSAIN didn't study. In total HUSSAIN studied 27 loci of which 19 are variable, in the present study 25 loci were analysed of which 18 were found to vary. When comparing the results these differences should be considered.

Sample size

Sample size is of utmost importance to detect the alleles present with different frequencies in the populations. As sample size increases the probability of detecting alleles present with low frequencies increases. A sample size of 8 seeds per tree, totalling 160 seeds per population used for the present studies offered a probability larger than 95 % of detecting alleles with a minimum frequency of 0.039 (GREGORIUS, 1980). HUSSAIN (1995) analysed seed of also 20 trees in six populations and of only 15 trees in the other six populations.

Genetic diversity

HAMRICK *et al.* (1981) have reported that conifers contain high levels of genetic diversities and are the most variable groups of species, as measured by isozyme analyses. That is, for the estimates of 20 species of conifers; the mean percentages of polymorphic loci, the numbers of alleles per locus and the expected heterozygosities are 67.7, 2.29 and 0.207, respectively.

In pines, a wide variation in percentage of polymorphic loci has been reported in the literature ranging from 0 % for *P. torreyana* to 100 % for *P. sylvestris*, *P. nigra*, *P. palustris*, *P. rigida* and *P. taeda* under the criterion that a locus was polymorphic if any allelic variant was observed (LEDIG, 1986). In *P. roxburghii*, 72 % of the investigated loci were polymorphic, HUSSAIN (1995) found 70 % when the above criterion was followed, whereas under 99 % criterion, the mean per cent polymorphic loci varied from 44.4 to 66.7 with an average value of 57.6 which is quite similar to the value (55 %) reported for *P. sibirica* (GONCHARENKO *et al.*, 1993).

The average number of alleles per locus of 1.65 found in this study and 1.49 found by HUSSAIN (1995) is within the range

(1.03 to 3.59) observed for six other pines with a mean value of 2.31 (ADAMS, 1983). The presence or absence of different allelic types gives an estimate of the representation of the total genetic variation of a species in any given population.

The mean effective number of alleles (allelic diversity) ranged from 1.087 (population Shalaghat) to 1.156 (population Shilly) and averaged at 1.130 which is slightly less but comparable with the mean value (1.260) reported for other conifer species (HAMRICK *et al.*, 1981).

Heterozygosity

Heterozygosity is directly applied as a measure of genetic variation (NEI, 1975; CROW, 1986). High levels of heterozygosity are important for survival over long life time of trees (GREGORIUS and ZIEHE, 1986), which is related with the low mortality of seedlings and plants (MEJNARTOWICZ and LEWANDOWSKI, 1994). Tree species in general are highly outcrossing as compared to herbaceous perennials and especially annuals (BAWA, 1974; FOWLER, 1965; SCHEMSKE and LANDE, 1985) and are the highly heterozygous plants, the average level of heterozygosity is twice of the herbaceous plants (HAMRICK *et al.*, 1979).

The average observed heterozygosities for the eight populations of *P. roxburghii* varied from 0.096 to 0.169 (*Table 1*) with an overall mean value of 0.147 which is in agreement to the values obtained by HUSSAIN (1995) ranging from 0.071 to 0.182 and a mean of 0.112. For *P. banksiana* DANZMANN and BUCHERT (1983) reported a mean heterozygosity of 0.146. LEDIG (1986) reported expected heterozygosities for 37 pines between 0.000 (*P. torreyana*) and 0.362 (*P. taeda*) with an average of 0.174 which is similar to the value He = 0.153 (*Table 1*) obtained for *P. roxburghii* in the present study.

Marginal populations usually show a lower heterozygosity than the central ones (BERGMANN and GREGORIUS, 1979; GURIES and LEDIG, 1982; YEH and LAYTON, 1979), this might be because marginal populations frequently owe their origin to colonising events and suffer from bottleneck effects as they emerge from a small number of founders. However, genetic diversity of marginal population Sulyali (from the lowest altitude) is well comparable with those of other populations whereas population Shalaghat (from the highest altitude) showed substantially lower diversity as compared to other populations. *P. roxburghii* forests are prone to forest fires and the low genetic diversity in population Shalaghat may be because of origin of this population from a small number of trees which may have survived after such fires in the past history of the population.

Fixation index

Significant fixation index values related to excesses of homozygotes at certain gene loci in *P. roxburghii* were observed as expected. The values reported for the populations studied by HUSSAIN (1995) were all negative and non significant. The significantly higher number of homozygotes found in three populations of the present study might be the outcome of inbreeding, Wahlund's effect (LI, 1955) and selection (ROBERDS and CONKLE, 1984; SPROULE and DANCIK, 1996). Though estimates of outcrossing in natural stands of pines have usually been higher (MUELLER, 1977) still inbreeding is not uncommon which produces more homozygotes than expected under Hardy-Weinberg expectations. SQUILLACE (1974) estimated natural self pollination frequency in different coniferous species varying from 2 to 40%. The percentage of selfing reported by YAZDANI *et al.* (1985) in the embryo stage of *P. sylvestris* ranged between 4 and 24% with an average of 11.8%. It is assumed that chances of inbreeding increase with the increase in isolation distance between the trees especially in wind pollinated species, which is reflected by the stock density of the stands. The stock densities of the stands under study were generally low varying from just 0.1–0.6 which could be the possible reason for inbreeding, especially selfing. In populations of *P. ponderosa* with low density, about 20% of the seeds were produced by selfing and genotypes in seeds exhibited deficiencies of heterozygotes to the extent of 17% (FARRIS and MITTON, 1984).

On the other hand, heterozygote excess may result from random union of gametes in case of different male and female frequencies (MUONA and SZMIDT, 1985) which is an important factor in conifer populations (MUELLER-STARCK *et al.*, 1983).

Heterogeneity in pollen pool produces different population structure than expected under Hardy-Weinberg expectations. However, it cannot be regarded as the reason for the observed significant deviation in the values of fixation index because pollen pool seems to be more homogeneous as reflected by small average value of subpopulation differentiation (delta = 0.024) for pollen cloud as compared to the value, delta = 0.051 for the ovules which is two times higher than the value observed for the pollen gene pool. The ovule gene pool consisted of twenty mother trees whereas number of the trees contributing to pollen cloud is unknown but probably higher. Gene pool homogeneity increases with the increase in number of contributing trees. Despite outcrossing behaviour of conifers, slight deficiencies of heterozygotes are frequently observed among embryos (DANCIK and YEH, 1983; DANZMANN and BUCHERT, 1983; FINS and LIBBY, 1982; GURIES and LEDIG, 1982; KNOWLES, 1984).

Genetic distance

The values of genetic distance (GREGORIUS, 1974) varied from 0.023 to 0.063 with a mean value of 0.043. Irregular changes in genetic distances among populations suggested that the variation was not clinal. A strong correlation between genetic and geographic distance is expected if only isolation by distance is responsible for the observed differentiation. The irregular changes in the genetic distance among the populations indicated that the allelic frequencies of the populations have been affected by some other factors may be differential selection under different edaphic and vegetation characteristics of the sites or mutation, including external environmental variables.

Cluster analysis reflected no clear grouping according to geographic location of the populations. However, two populations (Sulyali and Shalaghat) from the highest and the lowest altitude were the last to join the cluster. They were found to be different from other populations and also were not similar to each other. The results found by HUSSAIN (1995) showed similar results with low genetic distances and unclear clustering of the populations.

Genetic differentiation

Most of the studies on geographic variation in conifers have revealed high levels of genetic variation within populations and little differentiation among the populations (YEH and EL-KASSABY, 1980; Wheeler and GURIES, 1982; HIEBERT and HAMRICK, 1983; LOVELESS and HAMRICK, 1984; KIM *et al.*, 1994; MUELLER-STARCK, 1995; AGUNDEZ *et al.*, 1997). Usually over 90% variation in tree species is localised within populations (LEDIG, 1986). More than 10% of the total variation, however, has been reported to be found between populations for some pine species; *P. jeffreyi*, (FURNIER and ADAMS, 1986), *P. monticola*, (STEINHOFF *et al.*, 1983) and *P. radiata* (MORAN *et al.*, 1988). The mean level of diversity estimated for genus *Pinus* using GST is 6.5% (HAMRICK *et al.*, 1992).

Genetic variation within and between populations of different species depends on many factors including mating systems and generation length. LOVELESS and HAMRICK (1984) grouped species by mating system and found that between population variations were 0.523, 0.243 and 0.118 for autogamous, mixed and outcrossed species whereas according to generation length the values reported by them were 0.430, 0.262 and 0.077 for annuals, short-lived, and long-lived species, respectively.

Some studies conducted in the past on different tree species have detected microgeographical differentiation of genes or genotypic frequencies (LINHART *et al.*, 1981; KNOWLES, 1984; GREGORIUS *et al.*, 1986) and it has been assumed that this genetic substructuring is primarily the result of limited seed or pollen dispersal and mating of related individuals. SCHUSTER *et al.* (1989) reported that some populations of Limber pine (*P. flexilis* JAMES) on an elevational transect do not have overlapping pollination periods which may be a crucial factor contributing to the differentiation among the populations. Rapidly changing environmental conditions could produce asynchronous flowering, restricting gene flow between populations leading to high levels of differentiation among the populations.

Other studies have not detected any microgeographic patterns (GURIES and LEDIG, 1977; ROBERDS and CONKLE, 1984; NEALE and ADAMS, 1985; MORAN and ADAMS, 1989), suggesting that high levels of outcrossing and extensive gene flow by pollen and seed dispersal are the dominant forces in population structuring. Extensive pollen movement (KOSKI, 1970) and hence, high pollen counts at miles away from the source in wind pollinated tree species may explain low differentiation among the populations (SILEN, 1962; WANG *et al.*, 1960; LANNER, 1966; GRANT and MITTON, 1977). DIEBEL and FERET (1991) maintained that lack of barriers to gene flow prevented subpopulation differentiation in Fraser fir (*Abies fraseri* (Pursh) Poir).

Geographical distribution along with evolutionary history of a species generally explain the genetic differentiation within and among its populations (HAMRICK *et al.*, 1992; MUELLER-STARCK *et al.*, 1992)

About 96% of the total variation was found to be within the populations and only 4% among the populations of *P. roxburghii*. In his study, HUSSAIN (1995) estimated 94% within and 6% variation between populations. Similar values of variation, 96% within populations and 4% among populations have been reported for *P. contorta* (YEH and LEYTON, 1979) and *P. leucodermis* (MORGANTE and VENDRAMIN, 1990).

The populations of *P. roxburghii* were selected from the continuous distributional range of the species and hence large variation within populations and low differentiation among populations were expected. The small differentiation among populations at a gene locus may be on one hand ascribed to common descent of all populations and the time period since the separation of the populations was not sufficient for significant evolutionary modifications of the genetic structures e.g., as adjustment to different environmental conditions or strong gene flow among the populations. On the other hand similarity of genetic structures may be due to equal or parallel selection pressure. FINKELDEY (1993) argued that even with isozyme gene loci appears the acceptance of similar selection pressure in spite of apparent ecologically different environmental conditions. However, large differences were observed in allelic differentiation within gene loci among populations which does not permit to rule out the possibility of differential selection under different environmental situations. The differentiation observed for the studied gene loci may be reflection of differences in adaptation to rather different environments or to selection which took place during the past history of the populations (LUNDKVIST, 1979). For example, differences in soil moisture conditions or in vegetation characteristics of different population sites may lead to different selection pressures.

Generally it may be expected that marginal populations are more differentiated than the central ones. Peripheral populations are usually under more rigorous selective regime than their central counterparts. In the present study, the altitudinally marginal population (Shalaghat) showed the largest differentiation which confirmed the findings of BERGMANN and GREGORIUS (1979) who reported that in geographically marginal populations, the genetic variability of Norway spruce decreased. This population grows at the highest altitude and experiences low temperatures and snowfall more frequently than other populations.

Conclusions

Isozyme analysis has provided new information about the relative amounts of genetic variation present within and among eight populations of *P. roxburghii*. In the present study, large amount of genetic variation was found within populations and small portion of it among populations. Population Shilly generally showed highest values of genetic parameters but also significantly positive fixation index values exhibiting substantial inbreeding within this population. Populations Nihari and Shalaghat also revealed significant inbreeding. Among the other five populations showing non significant values of fixation index, populations Gagret and Nauni though had comparable numbers of alleles per locus yet less diversity and low hetrozygosity. Populations Banethi, Sulyali, and Aghar generally reflected higher values of genetic parameters.

Keeping in view these findings, management strategies should be focused on populations Sulyali Aghar, and Banethi for effective gene conservation, establishment of seed stands and breeding populations. These three populations are from lower, middle and upper ranges of the species distribution covering maximum geographic range in Himachal Pradesh.

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Genetic Variability in a Breeding Population of *Eucalyptus urophylla* S.T. Blake

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Resumo

Os programas de melhoramento florestal têm sofrido grande pressão para apresentar resultados em vista do aumento da demanda de produtos e derivados. Neste sentido, a aplicação prática de marcadores moleculares nestes programas tem sido vantajosa. Este trabalho procura incorporar a utilização do marcador RAPD na avaliação da variabilidade genética em uma população-base de *Eucalyptus urophylla* com os objetivos de avaliar sua base genética e compor um banco de dados moleculares desta população composta por 61 indivíduos das procedências Flores e Timor e uma variedade comercial local. Esta população foi avaliada através de 70 locos RAPD polimórficos. Os resultados mostraram que a população-base apresenta uma ampla base genética com média de similaridade entre indivíduos de 0,3168. A variedade comercial apresentou a menor média de similaridade (0,2885). Cruzamentos baseados em distância genética são propostos.

Abstract

Tree breeding programs have been under tremendous pressure to show results since the demand for the derived products has greatly increased. Molecular markers have been used in breeding programs in an attempt to evaluate the genetic bases of populations involved in breeding programs, identify hybrids and parental lines, etc. The aims of this study were to evaluate genetic variability of a base population of *Eucalyptus urophylla* using RAPD, to assess the genetic base of populations and to construct a molecular data bank. The base population consisted of 61 individuals of Flores and Timor provenance and a local commercial variety. Seventy polymorphic loci were analyzed. The mean genetic similarity was 0.3168 for the base population and the commercial variety showed the lowest similarity (0.2885). Crosses based on genetic distance were proposed.

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Key words: RAPD, genetic variability, molecular markers, *Eucalyptus urophylla*, genetic similarity, breeding population, crosses.

Introduction

In Brazil, research on silviculture started at the beginning of the 20th century, with the main objective of supplying wood in order to reduce the deforestation of natural forests. The new silviculture was based on exotic species, mainly *Pinus* and *Eucalyptus* species (FERREIRA and SANTOS, 1997).

Among several *Eucalyptus* species growing throughout Brazil, *Eucalyptus urophylla* is very important because it is mainly used for hybridization with *Eucalyptus grandis*. Nowadays, commercial programs are based on clonal plantations, where hybrids between these species, known as "Eucalyptus urograndis", are those most extensively utilized. These hybrids show uniformity, productivity and fixation of economic traits.

The use of molecular markers in breeding programs has been very attractive for plants breeders. Since they are based on DNA, molecular markers allow rapid assays of genetic parameters. RAPD has been one of the most used molecular techniques over the last years, mainly because it is simple, inexpensive, of rapid execution and highly polymorphic. Although this technique provides a low content of genetic information per locus and low transferability of data between different laboratories, RAPD has been widely applied to the genus *Eucalyptus* for clone identification (LANGE et al., 1993), to construct linkage maps (GRATTAPAGLIA et al., 1995; GRATTAPAGLIA and SEDEROFF, 1994), to estimate outcrossing rate (GAIOTTO et al., 1997), for fingerprinting (CHEN and FILLIPIS, 1996), and to distinguish individuals (NESBITT et al., 1995).

Nevertheless, additional basic research as important as that carried out in the cited studies should be performed with the aim of monitoring and helping programs of *Eucalyptus* breeding. Such studies should include, for example, assessment and monitoring of genetic variability within base populations and the establishment of heterotic groups crossing based on genetic distance using molecular markers. Molecular markers can also be applied to evaluate redundancy and deficiency of collections and to provide data on the efficiency of the collection, main-