Ann. For. Res. 54(1): 23-37, 2011

ANNALS OF FOREST RESEARCH
www.e-afr.org

Genetic diversity and differentiation through isozymes in natural populations of *Pinus wallichiana* A.B. Jacks (Blue Pine) in India

M. Bakshi, M. Konnert

Bakshi M., Konnert M. 2010. Genetic diversity and differentiation through isozymes in natural populations of *Pinus wallichiana* A.B. Jacks (Blue Pine) in India. Ann. For. Res. 54(1): 23-37, 2011.

Abstract. Eighteen natural populations of *Pinus wallichiana* A.B. Jacks. (Blue Pine) occurring in the Northwest Himalayas of India were studied on the basis of ten enzyme systems, representing 16 isozyme loci. Allele frequencies, genetic multiplicity, diversity and genetic differentiation values are presented. A marked allele frequency difference was found in different populations. The mean number of alleles per locus was 1.79, with a range from 1.56 to 2.06, and the gene pool diversity varied from 1.11 to 1.20. Out of 46 alleles 11 (23%) appear to be rare. Of the 16 isozyme loci scored, 14 (87.5 %) were polymorphic, based on the 99% criterion. Only the loci Pgm-A and Idh-A were found to be monomorphic in all the populations. The percentage of polymorphic loci ranged from 37.5% to 68.75% among populations. Nei's genetic distance ranged from 1.7% to 11%. The results show that natural populations of *Pinus wallichiana* in India contain an appreciable amount of genetic variation (P_{99} = 53.4%, H_o = 0.152, H_e = 0.145) comparable to other pines (on average P_{99} = 52, H_0 = 0.159, H_e = 0.159).
Keywords Isozymes, population, genetic diversity, polymorphism, genetic differentiation.

Author. Meena Bakshi (bakshim@icfre.org) - Plant Physiology (Botany Division), Forest Research Institute, Dehra Dun 248 006 Uttaranchal, India; Monnika Konnert - Bayerische Amt für Forstliche Saat und Pflanzenzucht Forstamtsplatz 1, D-83317 Teisendorf, Germany.
Manuscript received September 3, 2010; revised December 15, 2010; accepted December 19, 2010; online first February 3, 2011.

Introduction

Pinus wallichiana A.B. Jacks. (Syn. *P. griffithii* McClell), commonly known as Blue Pine is a highly variable species found throughout the temperate regions of the Himalayas, well distributed in Pakistan, India, Nepal, Bhutan, Tibet, China and Burma.

It is one of the most commercially important and widely distributed pine species in India. It

occurs in pure or mixed forests within latitudinal range of 25° N to 36° N, a longitudinal range of 68° E to 100° E and an altitudinal range of 1500 m to 3800 m along entire length of the Himalayas. In the northwest, it extends towards Jammu and Kashmir, Himachal Pradesh, Uttranchal and in the northeast in Sikkim, Assam, Meghalaya and Arunchal Pradesh states of India. It is highly valued for its timber, fuelwood, resins and turpentines and its resistance to blister rust caused by *Cronartium ribicola*.

Stands of Blue Pine are highly diverse throughout its range of distribution. Protection of its genetic resources and selection of quality wood planting material is highly appreciated in the frame of possible genetic divergence. For quality planting material, it is essential to use genetically improved material. The basic prerequisite for genetic improvement and the protection of genetic resources is the study of genetic variability. The wide adaptability of this species, under varying geographic, climatic and edaphic conditions is expected to be reflected in its genetic constitution, hence it offers great opportunity to study genetic variation in its diverse populations. Knowledge of the level and distribution of genetic variation both within and among populations facilitates the conservation of gene resources (Brown 1978, Miller & Libby 1991) and helps in developing strategies for conservation and tree improvement programmes.

For genetic variation studies, the choice of appropriate genetic markers assumes a great significance. Polymorphic isoenzymes, which are genetically controlled, have been used to describe and quantify the genetic variations of many forest tree species (Bergmann 1991, Muller-Starck 1991, Muller-Starck et al. 1992, Konnert 1995).

The present investigation was undertaken to analyze the genetic structure, the levels of genetic variation and differentiation among 18 natural populations of *Pinus wallichiana,* on the basis of 16 isozyme loci. This is the first population wide study of genetic variation through isozyme profiling in *Pinus wallichiana*.

Materials and methods

Geographic locations of the populations

Eighteen different populations of *Pinus wallichiana* were selected from North West Himalayan ranges, covering Himachal Pradesh and Uttaranchal states, with considerable geographic isolation. The location of the 18 populations (Figure 1) and the geographic data pertaining to them is shown in Table 1. Ten representative trees of approximately the same age were selected within each population, as per the methods adopted by FAO and Turnbull (1975). The selected trees were located at a minimum of 100 m apart to avoid narrowing genetic variation due to relatedness or inbreeding.

Sample materials and enzyme extraction

30 cones from different parts of the crown were collected from at least ten trees per population (300 cones/population). The cones were bulked together, dried and stored at 4-5° C before being analyzed. The seeds were manually extracted from cones. At the time of sampling, there was no significant pollution or pathogen damage. Embryos (30-50) isolated from dry seeds were used for analysis. In total 580 seeds (embryos) were analyzed for 10 enzyme systems. The sample material was homogenized in an extraction buffer (M Tris-Hcl, pH 7.0) containing 3% (w/v) polyvinyl pyrrolidone (PVP) and immediately prior to use 0.012% (v/v) 2-mercaptoethanol was added and the resulting homogenate was applied without any further purification on starch gels. The enzyme systems and gene loci investigated are listed in Table 2.

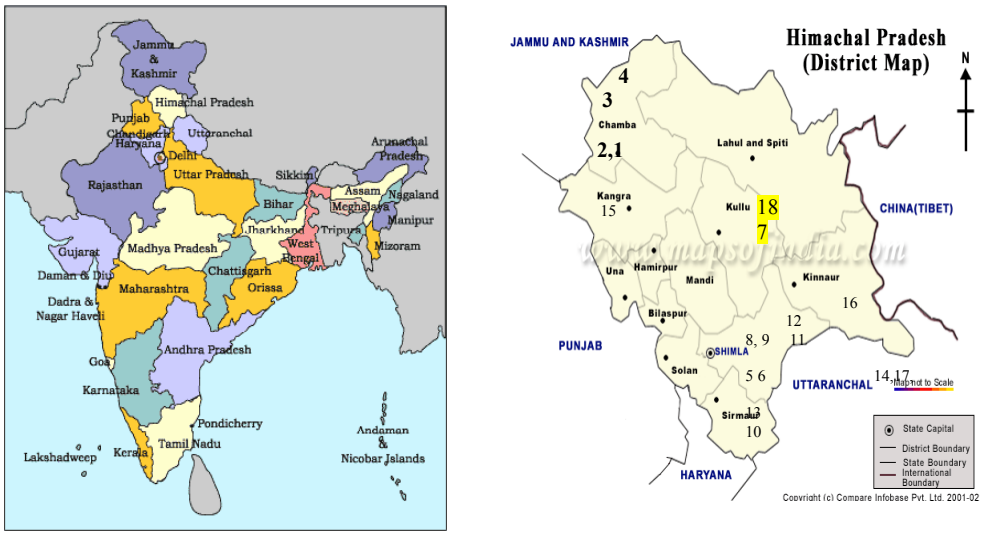

Figure 1 Location of populations (1-18)

Table 1 Geographic origin of the investigated populations (HP= Himachal Pradesh; UA= Uttaranchal)

Population	Location	Name of region	Latitude (N)	Longitude (E)	Altitude (m)
1	Sawai	Chamba (HP)	30° 20’	76° 26’	2900
2	Trehta	Chamba (HP)	30° 12’	76° 22’	2800
3	Bharmour	Chamba (HP)	32° 26’	76° 32’	2870
4	Tissa	Chamba (HP)	32° 49’	75° 50’	3000
5	Theog	Shimla (HP)	31° 12’	77° 20’	2300
6	Kotkhai	Shimla (HP)	31° 07’	77° 32’	2635
7	Sarahan	Kullu (HP)	31° 46’	77° 48’	2600
8	Tutu	Shimla (HP)	31° 12’	77° 51’	2100
9	Simla	Shimla (HP)	31° 10’	77° 36’	2400
10	Tikkar	Sirmaur (HP)	31° 29’	77° 16’	2000
11	Dodrakwar	Shimla (HP)	32° 03’	77° 57’	2800
12	Kasdaar	Shimla (HP)	31° 16’	77° 54’	2500
13	Chambikuper	Sirmaur (HP)	32° 05’	78° 20’	2665
14	Harsil	Uttarkashi (UA)	30° 46’	78° 44’	2533
15	Munder	Kangra (HP)	31° 40’	77° 15’	2305
16	Kalpa	Kinnuar (HP)	31° 35’	78° 15’	2768
17	Gangotri	Uttarkashi (UA)	31° 02’	79° 05’	2700
18	Nachan	Kullu (HP)	31° 32’	78° 08’	2250

Electrophoretic separation of isozymes

The isozyme analysis was performed by means of Horizontal Starch Gel electrophoresis (11.7% (w/v) Toronto Starch, 1.1% (w/v) Sucrose). The separation conditions include voltage (150-260 V), current (90-130 mA), running time (4-6 hours). The composition of buffer systems and the genetic interpretation of zymograms followed Muller-Starck & Starke

Table 2 Enzyme systems analzed, E.C. numbers and buffer systems used

Enzymes	E.C. No.	Scored loci	Buffer system employed
Fluorescent Esterases (Fest)	3.1.1.10	Fest- B	Ashton system
Glutamate-oxaloacetate transaminase (Got)	2.6.1.1	Got- A Got- B	Poulik system
Isocitric dehydrogenase (Idh)	1.1.1.42	Idh- A	Tris citric acid pH = 7.0
Leucine aminopeptidase (Lap)	3.4.11.1	Lap -B1	Ashton system
Malate dehydrogenase (Mdh)	1.1.1.37	Mdh -A4 Mdh- B Mdh-C Mdh-D	Tris citrate
Menadione reductase (Mnr)	1.6.99.2	Mnr-B	Poulik system
Phosphoglucose isomerase (Pgi)	5.3.1.9	Pgi-B1	Ashton system
Phosphoglucomutase (Pgm)	2.7.5.1	Pgm-A Pgm-B	Poulik system
6-Phosphogluconate dehydrogenase (6Pgd)	1.1.1.44	6Pgdh-A 6Pgdh-B	Tris citric acid pH = 7.0
Shikimate dehydrogenase (Skdh)	1.1.1.25	Sdh-A1	Tris citric acid pH = 7.0

(1993). Staining was performed according to Muller-Starck (1998).

Genetic parameters

Intra-and interpopulation computations were performed with MacGen. Software under SAS (Macgen - Stauber & Hertel 1997). On the basis of estimated allelic frequencies, genetic multiplicity, diversity and differentiation were computed in order to determine the extent of genetic variation within and among populations.

The parameters of multiplicity and diversity measures were: (i) *A/L* - average number of alleles per locus including all studied loci, (ii) *P* - percentage of polymorphic loci, computed on the basis of the 99% criterion (the frequency of most common allele was not greater than 0.99), (iii) *v* - gene pool allelic diversity also called effective number of alleles (Crow & Kimura 1986), whose average value per population is computed as the harmonic mean of single locus values, (iv) v_{gam} - hypothetical gametic multilocus-diversity (product of single locus values of *v*), (v) H_e - expected heterozygosity according to Hardy - Weinberg (Nei 1978), (vi) H_o - observed heterozygosity, (vii) *F* - Wright's fixation index (Wright 1922), computed in order to compare observed heterozygosities with panmictic expectations.

The contingency table Chi-square test (Snedecor & Cochran 1967) was used in order to estimate the heterogeneity between population distributions of allelic frequencies. The differentiation among populations was evaluated by: (i) *D* - allelic distance between pairs of population according to Gregorius (1974), (ii) δ = subpopulation differentiation (Gregorius & Roberds 1986), (iii) D_j - represents Gregorius genetic distance between each population and the remaining populations considered as a whole.

The computation was carried out with the assistance of specific SAS macros.

Results

Allele frequencies

Staining of 10 enzymes revealed gene products of 16 loci. Parameters of genetic variation were calculated on the basis of frequencies of 46 alleles (Table 3). Allele frequencies for the 18 populations and 16 investigated gene loci are listed also in Table 3. A marked allele frequency difference can be observed in different populations. Distinct differences in the allelic distribution are found at the gene loci Lap-B (B3 between 36.7-81.7%, B5 10-63.3%), Pgm-B (B3 33-80%, B4 20-67%), Pgdh A (A3 1-21.7%) and Sdh-A (A3 18-75.5%). Populations 6 and 9 are clearly different in frequency distribution at 3 loci viz. LapB, PgmB and SdhA. At Lap-B, with a frequency of 63.3% (population 6) and 58% (population 9) respectively, allele B5 has significantly higher values than in all other populations. At PgmB, 61% of populations have B3 as the most common allele while 39% showed B4 as the most common allele. Populations 6 and 9 distinguish themselves from other populations at the B4 locus having highest frequency of 63.3 and 67% respectively, at SdhA, A4 is the most common allele in 11 (61%) populations while in 7 (39%) of the populations, A3 is the most common allele. Low variability was shown by the gene locus PgdhB and PgmA.

A total of 46 alleles at 16 putative loci could be identified in the 18 populations analyzed. Of these, 46 alleles (23%) appear to be rare. Population 3 has 5 rare alleles, population 2 has 4, population 1 has 3, population 4, 13 and 17 have 2 while population 6, 9, 10, 11, 14 and 18 have one rare allele. However, in all the cases the frequency of rare was not greater than 3.6% which consequently excluded any sufficient contribution of these alleles to genetic differentiation. Population 10 is the only population with variation at MdhC, only population 4 has variation at PgmB2 and MdhB2 while only population 1 and 2 are variable at MdhA2.

Table 4 shows the Chi-Square values of heterogeneity among allelic frequency distributions in all populations calculated for all loci. The allelic frequencies of 8 out of 14 (57 %) loci were significantly heterogenous. There were no significant differences at the FestB, GotA, B, PgdhB, MdhA and MdhC loci.

Within population genetic variation

The data pertaining to six genetic indices viz. per cent polymorphic loci (*P*), average number of alleles per locus (*A/L*, 99% criterion), mean effective number of alleles (*v*), observed heterozygosity (H_o), expected heterozygosity (H_e) and Fixation index (*F*) are given in Table 5. Of the 16 isozymic loci scored 14 (87.5%) were polymorphic based on the 99% criterion. Only the loci PgmA and IdhA were found to be monomorohic in all the studied populations. The percentage of polymorphic loci ($P_{0.99}$) ranged from 37.5% (population 6 & 8) to 68.75 (population 1, 2, 4 and 13), with a mean of 53.4%. The number of identified alleles ranged from 24 to 33. The multiplicity within population ranged from 1.56 (population 6) to 2.06 (population 2). The mean number of alleles per locus (*A/L*) summed over all populations was 1.79. The minimum number of alleles was found in population 6 (24) and 8 (25). The value of gene pool diversity (***v***) varied from 1.1 (population 10) to 1.2 (population 2) with a mean of 1.14 and hypothetical gametic multiplicity diversity (***v***gm) ranged from 8.1 (population 4) to 29.6 (population 2) with a mean of 14.74. Interestingly, population 10 produced maximum average number of alleles per locus with maximum allelic diversity.

The mean observed heterozygosity (H_o) ranged from 11.3% (population 4) to 21% (population 6) with an average of 15.2%. The expected heterozygosity (H_e) ranged from 11.8% (population 10) to 18.8% (population 2) with a mean of 14.5%. Mean observed heterozygosity is slightly higher than the mean ex-

Table 3 Allele frequencies at the 16 analyzed enzyme loci for 18 populations of *Pinus wallichiana* in India

Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Fest- B	2	0.033	0.050	0.019	0.017	0.083	0.050	0.067		0.030	0.017	0.083	0.083	0.017	0.016		0.017	0.036	
	3	0.967	0.950	0.980	0.983	0.917	0.950	0.933	1.000	0.970	0.983	0.917	0.917	0.983	0.984	1.000	0.983	0.929	1.000
	4																	0.036	
Pgi-B	2	1.000	1.000	1.000	1.000	0.967	0.983	0.917	0.983	0.980	0.983	0.983	0.983	0.917	0.859	1.000	1.000	0.944	0.986
	3					0.033	0.017	0.083	0.017	0.020	0.017	0.017	0.017	0.083	0.141			0.056	0.014
Lap-B	2	0.033		0.078		0.017		0.017					0.017	0.017			0.017		
	3	0.700	0.667	0.667	0.733	0.700	0.367	0.650	0.817	0.390	0.783	0.586	0.750	0.650	0.710	0.672	0.603	0.518	0.414
	4	0.017	0.033		0.017	0.100			0.083	0.030	0.067	0.052	0.067	0.050	0.177	0.052	0.034	0.054	0.157
	5	0.250	0.300	0.255	0.250	0.183	0.633	0.333	0.100	0.580	0.150	0.362	0.167	0.283	0.113	0.276	0.345	0.429	0.429
Got-A	2	1.000	0.983	1.000	0.967	1.000	1.000	0.983	1.000	1.000	1.000	1.000	1.000	0.950	1.000	1.000	1.000	1.000	1.000
	3		0.017		0.033			0.017						0.050					
Got-B	2	0.017												0.033	0.017				
	3	0.933	0.883	1.000	0.950	0.967	1.000	0.967	1.000	0.990	1.000	1.000	1.000	0.967	0.948	0.983	1.000	1.000	0.929
	4	0.017			0.017										0.034	0.017			
	5	0.033	0.083		0.033	0.033		0.033											0.071
	7		0.033							0.010									
Pgm-A	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgm-B	2				0.017														
	3	0.633	0.533	0.794	0.467	0.600	0.367	0.700	0.800	0.330	0.633	0.465	0.483	0.550	0.672	0.429	0.379	0.518	0.571
	4	0.367	0.467	0.206	0.517	0.400	0.633	0.300	0.200	0.670	0.367	0.534	0.517	0.450	0.328	0.571	0.621	0.482	0.429
Pgdh-A	1			0.010								0.017		0.017					
	2	0.933	0.783	0.794	0.950	0.983	1.000	1.000	0.983	0.990	0.983	0.931	0.967	0.933	0.935	0.948	0.983	0.857	0.671
	3	0.067	0.217	0.167	0.050	0.017			0.017	0.010	0.017	0.052	0.033	0.050	0.064	0.052	0.017	0.107	0.071
	4			0.029														0.036	0.257
Pgdh-B	1	0.017	0.017	0.029															
	2	0.983	0.967	0.961	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	3		0.017	0.010															
Idh-A	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-A	2	0.017	0.017									0.033	0.033			0.016			
	3	0.983	0.983	1.000	1.000	1.000		1.000	1.000	1.000	1.000	0.967	0.967	1.000	1.000	0.948	1.000	1.000	1.000

Table 3 (continuation)

Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Mdh-B	2				0.017														
	3	0.917	0.933	0.961	0.967	0.967	0.717	0.950	0.750	0.740	0.933	0.883	0.800	0.917	0.969	0.862	0.983	1.000	1.000
	4	0.083	0.067	0.039	0.017	0.033	0.283	0.050	0.250	0.260	0.067	0.117	0.200	0.083	0.031	0.138	0.017		
Mdh-C	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.967	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	3										0.033								
Mdh-D	2	0.033	0.067																
	3	0.900	0.883	0.882	0.983	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.950	1.000	0.983	0.897	0.982	1.000
	4	0.067	0.050	0.118	0.017									0.050		0.017	0.103	0.018	
Sdh-A	2			0.049			0.067											0.071	
	3	0.350	0.367	0.569	0.183	0.217	0.650	0.467	0.500	0.750	0.300	0.313	0.567	0.533	0.344	0.232	0.328	0.661	0.300
	4	0.650	0.550	0.372	0.800	0.700	0.217	0.483	0.283	0.200	0.600	0.652	0.333	0.383	0.500	0.661	0.621	0.232	0.657
	5		0.017				0.017	0.017			0.067	0.017	0.017	0.033	0.031	0.036	0.052	0.018	0.029
	6		0.067	0.010	0.017	0.083	0.050	0.017	0.200	0.050	0.033	0.017	0.083	0.050	0.109	0.036			0.014
	7							0.017	0.017						0.016	0.036		0.018	
Mnr-B	2	0.067		0.039	0.033	0.050				0.010				0.033		0.017	0.034		0.043
	3	0.933	1.000	0.961	0.967	0.950	1.000	1.000	1.000	0.990	1.000	1.000	1.000	0.967	1.000	0.983	0.965	1.000	0.957

Table 4 Heterogeneity chi-square values compared for allele number based on allelic frequency distribution of the 18 populations

Locus	Chisquare	*DF*	*P*	Allele No. (*N*)
Fest-B	41.348	34	0.180	3
Pgi-B	50.708	17	0.001	2
Lap-B	203.408	51	0.001	4
Got-A	23.895	17	0.122	2
Got-B	90.432	68	0.036	5
Pgm-B	105.045	34	0.001	3
Pgdh-A	180.331	51	0.001	4
Pgdh-B	25.219	34	0.862	3
Mdh_A	26.484	17	0.066	2
Mdh-B	117.407	34	0.001	3
Mdh-C	11.939	17	0.804	2
Mdh-D	95.034	34	0.001	3
Sdh-A	302.224	85	0.001	6
Mnr-B	32.840	17	0.012	2

Table 5 Parameters of genetic diversity

Population	Total number of alleles (*N*)	Multiplicity *(A/L)*	*P*	Diversity *v*	*vgam*	$He_{\%}$	$Ho_{\%}$	*F*
01	32.0	2.00	68.7	1.16	15.28	15.8	15.0	0.051
02	33.0	2.06	68.7	1.20	29.60	18.8	12.9	0.314
03	31.0	1.93	56.2	1.16	15.60	15.4	17.3	-0.123
04	31.0	1.93	68.7	1.12	8.10	12.1	11.3	0.066
05	28.0	1.75	56.2	1.13	10.90	13.4	13.6	-0.015
06	24.0	1.56	37.5	1.14	14.00	14.2	21.0	-0.480
07	28.0	1.75	50.0	1.13	11.70	13.6	15.9	-0.169
08	25.0	1.56	37.5	1.12	9.90	12.2	11.3	0.074
09	27.0	1.68	56.2	1.13	11.50	13.5	19.2	-0.422
10	27.0	1.68	50.0	1.11	8.60	11.8	13.8	-0.170
11	28.0	1.75	50.0	1.15	14.90	14.9	15.8	-0.060
12	28.0	1.75	50.0	1.15	15.70	15.0	16.9	-0.127
13	32.0	2.00	68.7	1.17	20.70	16.7	17.9	-0.072
14	29.0	1.80	50.0	1.15	15.70	14.7	15.6	-0.061
15	29.0	1.80	56.2	1.14	13.40	14.3	13.7	0.042
16	27.0	1.68	50.0	1.13	11.50	13.2	14.6	-0.160
17	29.0	1.80	43.7	1.15	15.80	14.8	12.9	0.128
18	27.0	1.68	43.7	1.17	24.40	16.5	13.6	0.176
Mean	**28.6**	**1.79**	**53.4**	**1.14**	**14.74**	**14.5**	**15.2**	**-0.056**

Note: total number of alleles (*N*); *A/L* - mean number of alleles per locus, *P* - percentage of polymorphic loci, *v* and *vgam* - genetic diversity, H_e - expected heterozygosity according to Hardy-Weinberg, H_o - observed heterozygosity, *F* - fixation index.

pected heterozygosity. Among 18 populations for which values of H_o was calculated, in 11 populatioms, the average proportion of H_o was higher than expected under Hardy-Weinburg equilibrium, only in 7 populations, H_o were lower than H_e. Population 2 showed highest level of heterozygosity (H_e = 18.8%). Low heterozygosity (11.8%) was found for popula-

tion 10 which also had the lowest diversity. In comparison to other populations, population 2 is the most variable, while population 6 has the lowest level of genetic variability.

Excesses or deficiency of heterozygotes for each locus and each population was examined by means of Wright's Fixation Index, i.e. the inbreeding coefficient. Within populations the index ranged from –0.480 (population 6) to 0.314 (population 2), with an overall mean of –0.059 (6%), showing an excess of heterozygotes within populations. It means that about 94% of the genetic variation in *P. wallichiana* resided within the populations and only 6% among the populations. The fixation index values (inbreeding coefficient) are negative in twelve populations and positive for six populations. Positive *F* values reflected a deficiency of heterozygotes relative to Hardy Weinberg equilibrium. The maximum index value (0.314) was observed in population 2, indicating an excess of homozygotes. Population 8 with the lowest value (-0.480), showed an excess of heterozygotes. Significant deviations from Hardy Weinberg expectations were not detected.

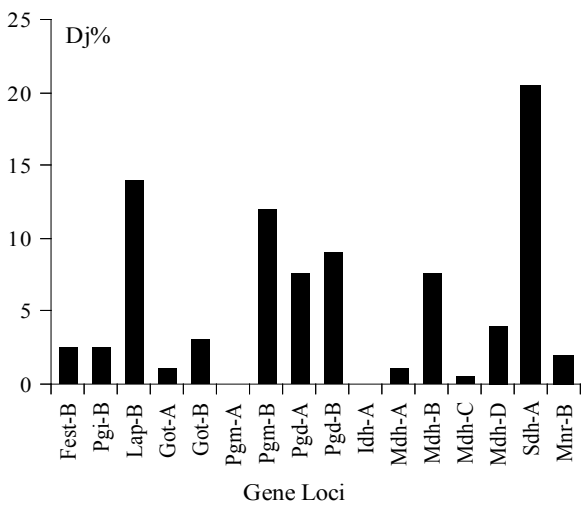

Figure 2 Differentiation between populations at 16 gene loci

Genetic differentiation among populations

The mean value of the subpopulation differentiation parameter (δ) estimated over all loci and populations was 5.7% (Table 6). This measure of genetic differentiation is based on the frequency distribution of alleles or genotypes, but here the frequencies of one population are compared against the weighted averages of the frequencies among the remaining populations. Hence, each population is considered to be a subpopulation, and the differentiation is measured by the genetic differentiation between it and the other 17 populations which are combined to form the respective complement population.

The values for genetic differentiation (δ) ranged between 2.6 (population 13) to 7.9% (population 6). The values calculated for the parameter D_j indicates that substantial variation exists among loci (0.4% to 20.2%). As illustrated from Figure 2, higher differentiation is found at the loci LapB, SdhA, MdhB and PgdA. The least differentiated populations over all loci are population 13 (D_j = 2.6%) and population 11 (D_j = 3.4%) while population 6 (D_j = 7.9%) and population 9 (D_j = 7.7%) are highly differentiated.

Genetic distance

Nei's unbiased genetic distance was calculated among 18 populations (Nei, 1978) and showed in Table 7. The genetic distance between pairs of population ranged from 1.7% to 10.8%, whereas more than half the population values lie between 3 to 5%. These values suggest only slight differences between populations at the gene pool level. Population 6 and 14 have very high distance values (10.8%) compared to other populations. A distance value of 10% is considered very high (Konnert 1995).

The largest genetic differences oc-

Table 6 Genetic differentiation among populations (δ values); D_j - differentiation values of single populations

Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Mean (δ)
	D_{j1}	D_{j2}	D_{j3}	D_{j4}	D_{j5}	D_{j6}	D_{j7}	D_{j8}	D_{j9}	D_{j10}	D_{j11}	D_{j12}	D_{j13}	D_{j14}	D_{j15}	D_{j16}	D_{j17}	D_{j18}	
Fest-B	0.003	0.017	0.018	0.021	0.052	0.017	0.034	0.038	0.007	0.021	0.052	0.052	0.021	0.021	0.038	0.020	0.037	0.038	0.028
Pgi-B	0.030	0.030	0.030	0.030	0.005	0.013	0.058	0.013	0.009	0.013	0.012	0.013	0.058	0.119	0.030	0.030	0.029	0.015	0.030
Lap-B	0.096	0.037	0.108	0.107	0.126	0.351	0.058	0.225	0.294	0.173	0.063	0.143	0.025	0.212	0.043	0.052	0.134	0.242	0.138
Got-A	0.007	0.011	0.007	0.028	0.007	0.007	0.011	0.007	0.007	0.007	0.007	0.007	0.046	0.007	0.007	0.007	0.007	0.007	0.011
Got-B	0.045	0.104	0.028	0.031	0.018	0.028	0.018	0.028	0.026	0.028	0.028	0.028	0.031	0.046	0.023	0.028	0.028	0.059	0.035
Pgm-A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pgm-B	0.087	0.020	0.257	0.090	0.051	0.197	0.157	0.263	0.235	0.087	0.092	0.073	0.002	0.128	0.131	0.183	0.036	0.021	0.117
Pgd-A	0.021	0.170	0.137	0.028	0.063	0.081	0.081	0.063	0.070	0.063	0.023	0.045	0.025	0.021	0.026	0.063	0.073	0.270	0.074
Pgd-B	0.014	0.030	0.036	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.009
Idh-A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-A	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.026	0.026	0.009	0.009	0.046	0.009	0.009	0.009	0.013
Mdh-B	0.015	0.032	0.062	0.084	0.068	0.198	0.050	0.163	0.173	0.032	0.021	0.110	0.015	0.070	0.044	0.085	0.103	0.103	0.079
Mdh-C	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.033	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.004
Mdh-D	0.074	0.092	0.099	0.014	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.027	0.032	0.013	0.084	0.013	0.032	0.041
Sdh-A	0.165	0.080	0.194	0.324	0.257	0.303	0.057	0.255	0.349	0.162	0.170	0.190	0.135	0.096	0.225	0.168	0.328	0.182	0.202
Mnr-B	0.051	0.019	0.022	0.016	0.034	0.019	0.019	0.019	0.009	0.019	0.019	0.019	0.016	0.019	0.001	0.017	0.019	0.026	0.020
Gene pool	0.039	0.041	0.063	0.049	0.045	0.079	0.037	0.070	0.077	0.043	0.034	0.046	0.026	0.049	0.040	0.047	0.051	0.063	0.057

Table 7 Estimates of Nei's (1978) genetic distance based on data from 16 loci, among populations

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	-	0.042	0.054	0.042	0.038	0.103	0.049	0.078	0.101	0.039	0.046	0.066	0.047	0.051	0.045	0.045	0.078	0.064
2		-	0.060	0.057	0.060	0.100	0.057	0.094	0.100	0.058	0.051	0.067	0.052	0.067	0.056	0.055	0.069	0.067
3			-	0.078	0.076	**0.103**	0.057	0.067	**0.105**	0.070	0.083	0.075	0.055	0.072	0.083	0.069	0.065	0.088
4				-	0.035	0.099	0.059	0.089	0.096	0.046	0.042	0.063	0.053	0.060	0.033	0.041	0.075	0.063
5					-	0.100	0.044	0.071	0.098	0.031	0.043	0.053	0.054	0.043	0.046	0.051	0.075	0.058
6						-	0.080	0.081	0.017	0.093	0.069	0.057	0.078	**0.108**	0.080	0.076	0.058	**0.102**
7							-	0.060	0.084	0.043	0.049	0.056	0.038	0.040	0.062	0.057	0.058	0.074
8								-	0.079	0.052	0.080	0.045	0.068	0.059	0.078	0.092	0.085	**0.107**
9									-	0.092	0.071	0.059	0.075	**0.106**	0.078	0.074	0.062	0.100
10										-	0.043	0.049	0.051	0.036	0.044	0.047	0.074	0.064
11											-	0.041	0.050	0.062	0.026	0.036	0.057	0.057
12												-	0.046	0.062	0.050	0.066	0.058	0.094
13													-	0.050	0.051	0.052	0.050	0.073
14														-	0.062	0.067	0.074	0.070
15															-	0.037	0.074	0.067
16																-	0.065	0.055
17																	-	0.067
18																		-

curred between populations 6 and 14 (DN = 10.8% which are distantly located. The smallest genetic difference was between 6 and 9 (DN = 1.7%) indicating the similarity of the populations gene frequencies and of note these populations are closely located.

Discussion

The aim of this work is to determine the degree of genetic variation within and among the indigenous *Pinus wallichiana* (Blue Pine) populations from Himachal Pradesh and Uttaranchal. Having analyzed 18 populations, we found that natural populations of *Pinus wallichiana* from India contain an appreciable amount of genetic variation, comparable to the mean values for the genus *Pinus* (on average - $P_{99} = 52$, $H_o = 0.159$, $H_e = 0.159$ - Goncharenko et al. 1989).

On the basis of the analysis of allelic frequencies, we inferred that the different populations studied have distinct differences in their genetic structures. Populations 6 (Kotkhai) and 9 (Simla) belonging to Shimla are clearly different in frequency distribution of alleles at three loci (LapB, PgmB and SdhA). The differences in allele frequencies among populations of forest tree species covering large geographic areas is essential for adaptation to variable environmental conditions. The highest values of genetic variation was observed in population 2 (Trehta), belonging to Chamba, while population 10 (Tikkar) showed the lowest level of genetic variability.

The mean number of alleles over all populations and loci found in this study was 1.79, against 1.69, obtained in *Pinus korianesis* (Krutovskii et al. 1990), 1.84 in *Pinus korianesis* (Potenko & Velikov 2001), 1.6 in *Pinus kesiya* (Myburg & Harris 1997), 1.8 in *P. brutia* (Panetsos et al. 1998) and 1.65 in *P. roxburghii* (Sharma et al. 2002). All the populations (1, 2, 3 and 4) belonging to Chamba showed rare alleles, whose frequency was not high. The presence of rare alleles could be due to deleterious mutations or may be evolution-

ary relics (Lindgren & Gregorious 1976). The distribution of high allelic frequency and the detection of rare alleles lead to the notion that populations present a degree of genetic differention. It is characteristic that the populations which possess rare alleles is within a typical *Pinus wallichiana* stands situated in Chamba.

The Chi-square values of heterogeneity among allelic frequency distributions in all populations calculated for all loci showed significant differences. The allelic frequencies of 8 out of 14 (57%) loci were significantly heterogenous. There were no significant differences at the FestB, GotA, B, PgdhB, MdhA and MdhC loci.

In Pines, a wide variation in percentage of polymorphic loci has been reported ranging from 0% for *P. torreyana,* to 100% for *P. sylvestris*, *P. nigra*, *P. palustris, P. rigida* and *P. teada* (Ledig 1986). The number of polymorphic loci ($P_{0.99}$) in *P. wallichiana* was 68%, compared to 47.9% in *Pinus korianesis* (Krutovskii et al. 1990), 84% in *Pinus koriensis* (Potenko &Velikov 2001), 57% in *P. brutia* (Panetsos et al. 1998) and 72% in *Pinus roxburghii* (Sharma et al. 2002). The mean effective number of alleles (allelic diversity) ranged from 1.11 (population Tikkar) to 1.20 (population Trehta) and averaged at 1.14, which is comparable with the mean value (1.26) reported for other conifer species (Hamarick et al. 1981).

Tree species are highly outcrossing and heterozygous in nature. Heterozygous individuals are, developmentally, more stable than homozygous counterparts (Lerner 1954, Soule 1979). Heterozygosity is a measure of genetic variation (Nei 1975, Crow 1986). The average observed heterozygosities for the eighteen populations of *P. wallichiana* varied from 0.113 to 0.21 (Table 3) with an overall mean of 0.152 which is in consonance to values (0.146) obtained in *P. banksiana* (Danzmann & Buchert 1983) and 0.147 (Sharma et al. 2002). The expected heterozygosity ranged from 0.118 to 0.188 with an average of 0.145 comparable to 0.153 in *P. roxburghii* (Sharma et al. 2002). Average values of expected (H_e = 14.5%) and observed heterozygosity (H_o = 15.1%) were low, the former is almost same as the average value of expected heterozygosity within populations estimated for gymnosperms (H_e = 15.1%) Hamrick et al. (1992) but is somewhat lower than the mean for 103 other isozymes in the family *Pinaceae* (H_e = 17.6%).

The fixation index values ranged from -0.480 to 0.314, with a mean of -0.056. The positive fixation index values related to excesses of homozygotes in 7 populations in this study could be explained to mating among closely adjacent individuals within a stand and selection against heterozygotes (Guries & Ledig 1982). Though the estimates of outcrossing in natural populations of pines is usually higher (Mueller 1977), still inbreeding is not uncommon in Pines. Natural self pollination frequency in different coniferous species ranged from 2 to 40% (Squillace 1974). The fixation index for 11 populations was negative, suggesting an excess of heterozygotes in these populations. Agreement of observed allelic frequency with Hardy-Weinberg expectations does not permit us to reject the null hypothesis of random mating and of absence of significant amount of genetic drift in this species.

When the inbreeding coefficient was estimated for loci, it became evident that there was a notable deficiency in heterozygotes in MnrB, PgdhC , GotA and to some extent in MdhD and SdhA. Low heterozygosity suggests positive mating among similar genotypes and selection of homozygote genotypes (Grunwald et al. 1986). Possibly favourable site conditions selected homozygous seedlings to survive and to become mature trees and thus enlarge the population.

Conifers contain high levels of genetic diversities and are the most variable groups of species (Hammarick et al. 1981). Most of the studies in conifers have shown high levels of genetic variation within populations and little differentiation among the populations (Kim et al. 1994, Mueller-Starck 1995, Agundez et

al. 1997, Sharma et al. 2002). About 94% of the total variation was found within the populations and only 6% among populations of *P. wallichiana*. Sharma et al. (2002) reported 96% of the total population within the populations and only 4% among populations in *P. roxburghii*. Similar values were also reported in *P. contorta* (Yeh & Leyton 1979). Geographic distribution, along with evolutionary history of a species generally explain the genetic differentiation within and among populations (Hamrick et al. 1992). The species having larger and more continuously distributed populations is likely to have more variation at population level and less variation between populations, and *Pinus wallichiana* seems to follow this trend.

In our studies, the populations of *P. wallichiana* were selected from the continuous distributional range of the species and, hence, large variation within populations and low differentiation among populations were obvious. Genetic variation within and between populations of different species apart from other factors is also dependent on the mating systems. High levels of outcrossing and extensive gene flow by pollen and seed dispersal are the dominant factors in population structuring. Low differentiation among populations may be due to extensive pollen movement miles away from the source in wind pollinated tree species. Lack of barrier to gene flow also prevens subpopulation differentiation. The small differentiation among populations may be ascribed to common descent of all populations and lapse of time to evolutionary separate the genetic architectures of the populations or it could be due to strong gene exchange among the populations. Large differences observed in allelic differentiation within gene loci, among populations, does not ignore the possibility of differential selection under different environmental situations.

Values of genetic distance (Nei 1978) ranged from 0.17 to 0.108, averaging 0.079, which is quite low. Genetic distances turned out to be the lowest in conifers (Goncharenko et al. 1989). The lowest level of genetic distance (DN = 1.7%) was found between population 6 and 9 belonging to Shimla and is apparently the result of intensive gene exchange between these populations. Low estimate of Nei's genetic distance confirm the close genetic relationship. The highest value of genetic distance was found between population 6 and 14 and 8 and 18 which are also distantly placed. The irregular changes in the genetic distance among the populations indicated that the allelic frequencies of the populations are governed by differential selection under different edaphic conditions of the site or mutations.

The changes that occurred in different populations could be explained as being caused by climatic natural selection. Our results in *Pinus wallichiana* reveal that the analyzed populations are as variable as other pine species natural populations studied by others in several European and Asiatic countries (Wang et al. 1991, Puglisi et al. 2000, Szmidt et al. 1996, Silin & Goncharenko 1996, Savolainen & Hadrick 1995). In general, the conifers present high levels of genetic variability and heterozygosity (Mitton 1983) and *P. wallichiana* is no exception from this fact.

The data obtained enable us to regard *P. wallichiana* as a highly polymorphic pine species. The high polymorphism in Blue Pine lead to physiological plasticity of the species enabling it to adapt to variable climatic conditions. Our results in different populations of blue pine show quite high genetic diversity in population 2 (A/L = 2.06, v = 1.2) and population 13 (A/L = 2.0, v = 1.17) belonging to Chamba and Sirmaur respectively. Population 2 (Trehta) revealed highest values of genetic parameters, but also, the significantly positive fixation index values indicate sufficient inbreeding within this population. Among other population 13 also revealed ample diversity with high heterozygosity. Therefore, these forests should be treated as a biogenetic resources, that require protection. Populations 6 and 9 have dif-

ferent allelic architecture, hence may be good for breeding programmes. The observed large within population variation and high outcrossing rate are encouraging for potential breeding applications and for conservation.

Conclusion

Pinus wallichiana A.B. Jacks. is one of the most commercially important and widely distributed pine species in India, well known for resistance to white pine blister rust disease. The species enjoys wide adaptability under varying geographic, climatic and edaphic conditions which is reflected in its genetic constitution.

Genetic diversity study through isoenzymes in this species revealed ample genetic variation among 18 populations selected in Himachal Pradesh and Uttranchal states of India, which is comparable to other gymnosperms. The present study revealed large amount of genetic variation within populations and small portion of it among populations. The findings revealed ample diversity in population 2 (A/L = 2.06, v = 1.2) and population 13 (A/L = 2.0, v = 1.17) belonging to Chamba and Sirmaur respectively. Therefore, it is recommended to conserve these forests as biogenetic resources. Populations 6 and 9 have different allelic architecture, hence may be a potential source for future breeding strategies. Such a wide diversity explored through isozyme analysis in *Pinus wallichiana* will be beneficial for future breeding programmes and improvement of the species.

Acknowledgments

The authors wish to thank D.A.A.D. for extending financial support to run this project, to Dr Albrecht Behm to grant permission to work in the Institute and to Mr Rolf, Carina, Huber and Evelyn for their excellent technical assistance.

References

Agundez D., Alia R., Degen B., Wuehlisch G., 1997. Genetic variation of Aleppo pine (*Pinus halepensis* Mill.) in Spain. Forest Genetics 4(4): 201-209.

Bergmann F., 1991. Isozyme gene markers. In: G. Muller-Starck & M. Ziehe (eds.), Genetic variation in European populations of forest trees. J.D. Sauerlanders, Frankfurt/Main. pp. 67-79.

Brown A.H.D., 1978. Isozymes, plant population genetic structure and genetics conservation. Theoretical and Applied Genetics 52: 145-157.

Crow J.F., Kimura M., 1970. An introduction to population genetic theory. Harper and Row Publishers, New York, Evanston, London.

Crow J.F., 1986. Basic concepts in population, quantitative and evolutionary genetics. W. H. Freeman and Comp., New York.

Danzmann R.G., Buchert G.P., 1983. Isozyme variability in central Ontario jack pine. In: Eckert R.T.: Proceedings of the Twety-eighth Northeastern Forest Tree Improvement Conference. Institute of Natural and Environmental Pesources, University of New Hampshire, Durham, NH, pp. 232-248.

Gillet Elizabeth M., 1998. Genetic Structures from Electrophoresis Data (GSED) Users Manual http:/www.uni-forst.gwdg.de/forst/fg/software.htm.

Goncharenko G.G., Pudutov V.E., Potenko V.V., 1989. Guide to conifer species research by isozyme methods. Byelorussian Forest Research Institute, Gomel.

Gregorius H.R., 1974. Genetischer Abstand zwischen Populationen. I. Zur Konzeption der genetischen Abstandsmessung. [Genetic distance between populations. I. On the concept of genetic distance measurement]. Silvae Genetica 23: 22-27.

Gregorius H.R., Roberds J.H., 1986. Measurement of genetical differentiation among subpopulations. Theoretical and Applied Genetics 71: 826-834 .

Grunwald C., Schiller, G., Conkle, M.T., 1986. Isozyme variation among native stands and plantations of Aleppo pine in Israel. Israel Journal of Botany 35: 161-174.

Guries R.P., Ledig F.T., 1981. Genetic structure of populations and differentiation in forest trees. In: Conkle, M.T. (ed.) Proceedings of the symposium on isozymes of North American forest tree and forest insects, U.S. Forest Service, General Technical Report PSW-48, pp. 42-47.

Hamarick J.L., Mitton J.B., Linhart Y.B., 1981. Levels of genetic variation in trees : influence of life history characteristics. In: Conkle M. T. (ed.) Proceedings of symposyum on isozymes of North American forest trees and forest insects. U.S. Forest Service, General Technical Report. PSW-48, pp. 35-41.

Hamrick J.L., Godt M.J.W., Sherman B.S.L., 1992. Factors influencing levels of genetic diversity in woody plant species. New Forests 6: 95-124.

Kim Z., Lee S., Lim J., Hwang J., Kwon K., 1994. Genetic

diversity and structure of natural populations of *Pinus koriensis* (Sieb. Et Zucc.) in Korea. Forest Genetics 1: 41-49.

Konnert M., 1995. Investigations on the genetic variation of beech (*Fagus sylvatica* L.) in Bavaria. Silvae Genetica 44: 5-6.

Krutovskii K.V., Politov D.V. Altukhov Y.P., 1990. Genetic differentiation between Eurasian Cedar pines for isozyme loci. Soviet Genetika 26: 694-707.

Ledig F.T., 1986. Heterozygosity, heterosis and fitness in outbreeding plants. In: Soule, M (ed.) Conservation biology: the science of scarcity and diversity. Sinauer associates, Sunderland, Mass, pp. 77-104.

Lerner I.M., 1954. Genetic Homeostasis. Wiley, New York.

Lindgren D., Gregorius H., 1976. Inbreeding and coancestry. In: Proceedings of IUFRO Joint Meeting on advanced genetic breeding, Bordeaux, France, pp. 49-71.

Macgen-Stauber H., 1997: http;/www.mol.schuttle.de/wspc/genetic 1. htm) and with the GSED (Gillet 1998).

Macgen S. & Hertel, 1997. http://www.mol.schuttle.de/wspc/genetik I.htm).

Millar C.I., Libby W.J., 1991. Strategies for conserving clinal ecotypic and disjunct population diversity in widespread species. In: Falk D.A. and Holsinger, K. E (eds.), Genetics and Conservation of rare plants, Oxford University Press, pp. 149-170.

Mitton J.B., 1983. Conifers. In: Tanskley. S. D., Orton T. J. (eds.). Isozymes in plant genetics and breeding Part B. Elsevier Sci. Publ. B. V. Amsterdam, pp. 443- 472.

Müller G., 1977. Untersuchungen uber die naturliche Selbstbefruchtung in Bestanden der Fichte (Picea abies (L.) Karst.) und Kiefer (*Pinus sylvestris*). [Studies on the natural self-fertilization in stands of spruce (*Picea abies* (L.) Karst) and pine (*Pinus sylvestris*)]. Silvae Genetica 26: 207-217.

Müller-Starck G., 1991. Survey of genetic variation as inferred from enzyme gene markers. In: G. Muller-Starck, and Ziehe M. (eds.), Genetic variation in European populations of forest trees. J. D. Sauerlanders, Frankfurt/Main. pp. 20-37.

Müller-Starck G., Baradat Ph., Bergmann F., 1992. Genetic variation within European tree species. New Forests 6: 23-47.

Müller-Starck G., 1995. Genetic variation in high elevated populations of Norway spruce (*Picea abies* (L.) Karst.) in Switzerland. Silvae Genetica, 44: 356-362.

Müller-Starck G., 1998. Isozymes. In: Karp, A. Isaac P. G., Ingram D., (eds.) Molecular tools for screening biodiversity: plants and animals. Chapmann and Hall, London, pp.75-81.

Müller-Starck G., Gregorius H.R., 1986. Monitoring genetic variation in forest tree populations. In: Proceedings of the 18th IUFRO World Congress, Division 2 Vol. II, 7-21 September, Ljubljana pp. 589-599.

Myburg H., Harris S.A., 1997. Genetic variation across the natural distribution of the South East Asian Pine, *Pinus kesiya* Royle ex Gordon (Pinaceae). Silvae Genetica, 46(5): 295-300.

Nei M., 1975. Molecular population genetics and evolution. North-Holland Pub. Co. Amsterdam, North Holland.

Nei M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

Panetsos K.P., Aravanopoulos F.A., Scaltsoyiannes A., 1998. Genetic variation of *Pinus brutia* from islands of the Northeastern Aegean Sea. Silvae Genetica 47(2-3): 115-120.

Potenko V.V., Velikov A.V., 2001. Allozyme variation and mating system of coastal populations of *Pinus koraiensis* Sieb. et Zucc. 24cc. in Russia. Silvae Genetica 50(3-4): 117-122.

Puglisi S., Attolico M., 2000. Allozyme variation in natural populations of the Italian range of *Pinus sylvestris*. L. Forest Genetics 7(3): 221-232.

Savolainen O., Hedrick P., 1995. Heterozygosity and fitness: no association in Scots pine. Genetics 140: 755-766.

Sharma K., Degen B., Wuehlisch G. von Singh, N. B., 2001. Allozyme variation in eight natural populations of *Pinus roxburghii* Sarg. in India. Silvae Genetica 51(5): 246-253.

Shaw G.R., 1914. The genus *Pinus*. Journal of Arnold Arboretum Publications 5.

Shaw G.R., 1924. Notes on the genus *Pinus*. Journal of Arnold Arboretum 5: 225-227.

Silin A.E., Goncharenko G.G., 1996. Allozyme variation in natural populations of Eurasian Pines IV. Population structure and genetic variation in geographically related and isolated populations of *Pinus nigra* Arnold on the Crimean Peninsula. Silvae Genetics 45(2-3): 67-75.

Snedecor G.W., Cochran W.G., 1967. Statistical methods. The Iowa State University Press, Ames, Iowa.

Soule M.E., 1979. Heterozygosity and developmental stability: another look. Evaluation 33: 396-401.

Squillace A.E., 1974. Average genetic correlations among offspring from open pollinated forest trees. Silvae Genetica 23: 149-156.

Szmidt A.E., Wang X.R., Lu M.Z., 1996. Empirical assessment of allozyme and RAPD variation in *Pinus sylvestris* (L.) using haploid tissue analysis. Heredity 76: 412-420.

Wang X.R., Szmidt A.E., Lindgren D., 1991. Allozyme differentiation among populations of *Pinus sylvestris* (L.) from Sweden and China. Hereditas 114: 219-226.

Wright S., 1922. Coefficients of inbreeding and relationship. American Naturalist 56: 330-338.