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Genetic Variability in *Pinus roxburghii* Sarg. Revealed by RAPD Markers

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ABSTRACT

RAPD (Random Amplified Polymorphic DNA) markers were used to reveal genetic variation in 55 geographically distinct populations of *Pinus roxburghii* of the Himalayan region, covering the entire natural range of distribution of the species in India. Populations vary between the longitude range of 71° 1′ N to 92° 23′ E, latitude 27° 19′ N to 33° 22′ N within an altitudinal range of 520 m to 2205 m. A total of 50 decamer primers were used, out of which twenty primers were selected based upon the polymorphism and reproducibility of results. Primers generated a total of 231 amplification products, out of which 191 amplicons were polymorphic. Amplification products obtained per primer ranged from as low as 5 to a maximum of 18, with an average of 11.55. The extent of polymorphism varied with the primer. Divergence in respect of percent polymorphism ranged from 76 to 100% with an average of 84% in the species. The similarity coefficient ranged from 0.36 to 0.78 and the genetic distance varied from 0.23 to 0.53 between the populations. Based on the UPGMA dendrogram and genetic similarity pattern, the populations of *P. roxburghii* were grouped into five major clusters. Genetic similarity analysis showed considerable variation among the populations ranging from highest similarity between the populations of Una, Badasar (Himachal Pradesh; HP) and Jamta (HP); Una, Badasar (HP) and Manikaran (HP) with 78% similarity. Highest dissimilarity was observed between the populations of Gaucher (Uttarakhand) and Akhnoor (Jammu and Kashmir) with only 36% similarity (64% dissimilarity). The results reveal that there is a fair amount genetic variability in the existing forests of Himalayan chir pine (*P. roxburghii*) and as such the information is valuable with regard to initiate conservation and genetic improvement efforts in this species.

Keywords: cluster, genetic variation, polymorphism, population, RAPD, similarity index
Abbreviations: HP, Himachal Pradesh; **JK**, Jammu and Kashmir; **NE**, North-East; **RAPD**, random amplified polymorphic DNA; **UK**, Uttarakhand

INTRODUCTION

Pinus roxburghii Sargent (Chir pine or longneedle pine), is a gregarious, fire resistant and the most important pine species amongst six indigenous pine species of India. It is a precious timber-resin resource, is native to the outer range and principal valleys of the Himalayas from Afghanistan to Arunachal Pradesh in northeastern India between 450 and 2300 m altitude, where the full force of the monsoon is felt. Its distribution in India is confined to Jammu and Kashmir (JK), Himachal Pradesh (HP), Uttarakhand (UK), Sikkim, West Bengal and Arunanchal Pradesh. It covers very large areas as pure forests and also with other species, particularly at its upper and lower limits. The species is a source of a variety of goods for the society namely timber for house construction, packing cases, fuel wood and charcoal, needles for cattle feeding and manure, fuel for baking terra cotta articles etc. Resin industry of the country is dependent mainly on this species. Its commercial value is enhanced by the fact that it is adapted to a wide range of soil and climatic conditions. There is an immense scope for genetic improvement in chir pine due to its wide range of distribution in the western and central Himalaya (Sehgal *et al*. 1995). However, being highly cross-pollinated and its long rotation period have placed most of the tree improvement programs in the initial stage.

The extensive utility (Le Maitre 1998) and ecological significance (Richardson and Rundel 1998) of *Pinus* has made it the focus of numerous molecular evolutionary studies (Price *et al.* 1998; Liston *et al*. 2003). The genetic structure of populations defines how the natural variation in populations can be used to detect and map genomic areas of functional importance. An understanding of the amounts, pattern and associations of genetic variation is a major goal of population genetics studies. Such knowledge is fundamental not only for tests of evolutionary hypothesis, but also for effective germplasm improvement and conservation strategies (Dong and Wagner 1993). The development of rapid molecular genetic analysis with gene markers and their acceptability throughout the world provide ample scope in their application to *Pinus* tree improvement programs. Pine represents a model system for population and evolutionary genetics investigations and DNA based analysis enables to analyse the variation in genetic material itself. A maternally inherited mitochondrial polymorphism in pine features abundant population variation (Dong and Wagner 1993) while paternally inherited cpDNA insertion/deletion polymorphism displays relatively little differentiation among the specific population (Wheeler and Guries 1982).

Random amplified polymorphic DNA (RAPD) technique has been shown to be effective in identifying useful polymorphism in both repetitive DNA and low copy DNA sequences (Williams *et al.* 1990). The use of RAPD assay to identify genetic variation is preferred over the conventional morphological and biochemical markers since these are completely devoid of effects of environment thus making them highly reliable (Rajagopal *et al.* 2000). RAPDs and other molecular markers have been extensively used in forest tree breeding and molecular ecology, to identify somaclonal variation, to assess biodiversity as a tool to aid the preservation of valuable germplasm (Teixeira da Silva *et al.* 2005). Knowledge of existing information on the *Pinus* genus related to molecular-based genetic analysis pro-

Received: 12 September, 2009. Accepted: 29 January, 2010.

Original Research Paper

vides an opportunity to study the variability and diversity in *P. roxburghii* which is to date unexplored. The main objectives of the study were to analyze and document population variation in *P. roxburghii* through RAPD markers and establish genetic relationship between the provenances/ populations occurring in the Himalayas.

MATERIALS AND METHODS

Foliage/needles of fifty-five geographically distinct small populations were collected from natural range of distribution of *P. roxburghii* in India. Ten trees were chosen from each population for the collection of foliage/needles. Selection of trees for collecting samples was done randomly ensuring representation of all classes of trees in the sample. Trees considered for collection were well spaced and separated from each other by at least 100 m. Needles from each tree belonging to a population were collected and tagged separately. Samples were brought to the laboratory and stored at -80°C. The detail regarding the geographical locations of the populations is given in **Table 1**.

Table 1 Details of various provenances of chir pine (*P. roxburghii*).

Index no.	Provenance/origin	Long. (° ′) E	Lat. (° ′) N	Altitude (m)
P1	Akhnoor (JK)	74.42	32.55	1225
P2	Augustmuni (UK)	79.3	30.18	762
P3	Bahli,Taklekh (HP)	77.37	31.23	1300
P4	Bakloh (HP)	75.52	32.44	1350
P5	Banethi (HP)	77.16	30.38	1,370
P6	Banikhet (HP)	75.4	32.4	1400
P7	Barkot (UK)	78.14	30.48	1,500
P8	Bhowali (UK)	79.33	29.23	1,706
P9	Bhuntar (HP)	71.1	32.9	1,100
P10	Birahi (UK)	79.9	30.9	1,219
P11	Brindavan Nurpur (HP)	76.33	31.12	575
P12	Chamba Tikri (HP)	76.13	32.46	923
P13	Chiwa (UK)	77.5	30.57	1,500
P14	Danoghat (HP)	76.12	30.81	1,150
P15	Dharamigad (UK)	77.38	30.26	913
P16	Dharampur (HP)	77.14	30.54	1,500
P17	Dharamsala (HP)	76.23	32.7	1,250
P18	Dharasu (UK)	78.17	30.37	650
P19	Dirang, Jorhat (NE)	92.15	27.45	1350
P20	Gangolihat (UK)	80.02	29.4	1,100
P21	Gaucher (UK)	79.11	30.17	729
P22	Hamirpur (HP)	76.3	32.12	850
P23	Jamata (HP)	77.18	30.36	932
P24	Jarmola (UK)	78.6	30.57	1,828
P25	Jumhar,Chamba (HP)	76.1	32.44	1100
P26	Katra (JK)	74.55	32.58	520
P27	Kiarala (HP)	77.38	30.55	1,200
P28	Kothar (HP)	76.4	30.5	1,150
P29	Kunihar (HP)	76.36	30.52	1,200
P30	Mandi (HP)	76.55	31.43	760
P31	Manikaran (HP)	77.21	32.2	1,737
P32	Mastgarh (HP)	76.1	32.23	700
P33	Merwa (HP)	77.18	30.5	1,250
P34	Mohand (UK)	77.17	30.18	675
P35	Nadaun (HP)	76.6	32.2	650
P36	Nafra (NE)	92.23	27.19	1200
P37	Nandprayag (UK)	79.5	30.7	1,200
P38	Narang (HP)	77.25	30.2	1,300
P39	Naushera (JK)	74.14	33.09	1200
P40	Palampur (HP)	76.9	31.4	1,240
P41	Pharsula (UK)	78.44	29.49	1,300
P42	Pokhra (UK)	78.5	29.56	1,350
P43	Rajgarh (HP)	77.19	30.51	1,400
P44	Rajouri (JK)	74.16	33.22	668.2
P45	Ramnagar (JK)	75.18	32.48	520
P46	Ranikhet (UK)	79.25	29.38	1,800
P47	Sabathu (HP)	76.59	30.58	1,400
P48	Suket (HP)	77.6	31.23	1,830
P49	Supin Sankri (UK)	78.5	31.7	1,800
P50	Taradevi (HP)	77.9	31.06	2205
P51	Thadiyar (UK)	77.55	30.58	1,200
P52	Thalisian (UK)	79.2	30.1	1,500
P53	Theture (UK)	78.11	30.3	1,500
P54	Trisula (UK)	79.15	30.12	1,800
P55	Una, Badsar (HP)	76.16	31.28	1,200

DNA extraction

Genomic DNA was extracted from young needles using the protocol described by Stange *et al.* (1998) with slight modification. Leaf material (250 mg) was ground using a mortar and pestle to fine powder by liquid N_2 and suspended in 1 ml of extraction buffer (2% CTAB, 1.42 M NaCl, 20 mM EDTA, 100 mM Tris HCl, pH 8.0, 2% w/v PVP40, 5 mM ascorbic acid, and freshly added 3 µl β-mercaptoethanol) added to 2 ml centrifuge tubes. The mixture was incubated at 60°C for about 30 min. Following incubation, 500 µl of chloroform: isoamyl alcohol (24: 1) was added, mixed to form an emulsion and centrifuged at 14,000 rpm for 10 min. The top aqueous phase was taken out in a fresh autoclaved centrifuge tube, and DNA was precipitated by addition of equal volume of cold iso-propanol. The precipitated DNA was precipitated by centrifugation at 10,000 rpm for 10 min. The DNA pellet was washed with wash buffer (76% ethanol, 10 mM ammonium acetate) and dried. The pellet was further treated with 5 µl RNase A (10 mg/l). The pellet was incubated and washed with cold 70% ethanol, dried and resuspended in 100 µl of TE buffer (10 mM Tris HCl; 1 mM EDTA; pH 8.0). The isolated DNA was quantified and all the samples were brought down to a uniform concentration of 5 ng/µl to be used as template DNA for Polymerase Chain reaction. The isolated DNA from 10 trees of each population was bulked uniformly and a composite sample representing the population was made.

RAPD analysis

For RAPD marker analysis, a total of 50 decamer primers from the list given by Mosseler *et al*. (1992) and Operon (QIAGEN Operon, 1000 Atlantic Avenue, Alameda, CA 94501, USA) were initially used in the present study, of which twenty primers were selected based upon their reproducibility and polymorphic nature for screening of germplasm. The detail and nucleotide sequence of the RAPD primers is given in **Table 2**. Amplification with each primer was repeated at least twice in order to ensure reproducibility and only those bands, which occurred consistently, were considered for the analysis.

PCR for RAPD marker amplification was performed on a Mastercycler Gradient PCR system (Eppendorf, Germany), incorporating 5 ng genomic DNA to a 25 µl reaction mixture containing 1X reaction buffer (100 mM Tris pH 9.0; 500 mM KCl; 0.1% gelatin), 2.0 mM $Mgcl_2$, 200 µM (0.2 mM) of each dNTPs (Bangalore Genei, Bangalore, India), 0.4 µM of each primer, 1 unit of *Taq* DNA polymerase (Bangalore Genei, Bangalore, India). The PCR amplification programme consisted of: initial denaturation at 94°C for 3 min, 45 cycles each of 94°C for 1 min, 37°C for 1 min, 72°C for 2 min and a final extension step at 72°C for 7 min. The amplified fragments were resolved and visualized on 1.5% agarose gel stained with ethidium bromide (0.5 µg/ml) at 90V for 3.0–3.5 h.

Statistical analysis

Each amplified product was scored across all the populations for its presence or absence. The RAPD profiles were analysed through GENE PROFILER (Scanalytics, Inc., 8550 Lee Highway, Fairfax, VA 22031, USA) software to score and size the separated fragments. The Data matrix obtained for presence or absence of RAPD bands was analyzed according to Nei and Li (1979) definition of genetic similarity, i.e Sij = 2a/(2a+b+c) where sij is the similarity coefficient between two individuals (i and j) , 'a' is number of bands present in j and absent in i. In order to analyze the relatedness among the population the Unweighted pair Group Method with Arithmetic Averages (UPGMA) based dendrogram was constructed using NEI and Li genetic distance matrix (NEI and Li, 1979) using TREECON (Scanalytics, Inc., 8550 Lee Highway, Fairfax, VA 22031, USA) program.

Table 2 List of primers sequences with their percent polymorphism in *Pinus roxburghii*.

Name of primer	Base sequence (5′-3′)	№ of amplification products	№ of polymorphic bands	Polymorphism (%)
Mosseler-188	GCT GGA CAT C	6	6	100
Mosseler-198	GCA GGA CTG C	9	9	100
Mosseler-119	ATT GGG CGA T	5	4	80
Mosseler-122	GTA GAC GAG C	13	10	77
Mosseler-184	CAA ACG GCA C	17	14	82
Mosseler-186	GTG CGT CGC T	9	7	78
Mosseler-191	CGA TGG CTT T	9	8	89
Mosseler-199	GCT CCC CCA C	15	13	87
OPA-02	TGC CGA GCT G	9	7	78
OPA-03	AGT CAG CCA C	15	13	87
OPA-08	GTG ACGTAG G	11	9	82
OPA-09	GGG TAA CGC C	9	7	78
OPA-10	GTG ATC GCA G	11	9	82
OPA-01	CAG GCC CTT C	13	10	77
OPA-07	GAA ACG GGT G	13	11	85
Mosseler-29	CCG GCC TTA C	10	8	80
Mosseler-31	CCG GCC TTC C	18	15	83
Mosseler-147	GTG CGT CCT C	17	13	76
Mosseler-156	GCC TGG TTG C	9	7	78
Mosseler-182	GTT CTC GTG T	13	11	85
Total		231	191	82.68 (mean)

RESULTS AND DISCUSSION

There was considerable variation in the level of polymorphism among the populations of *P. roxburghii*. The primers generally amplified a different number of markers and demonstrated a comparable degree of polymorphism across the populations. Amplification of total genomic DNA from different sources produced a total of 231 amplified bands, out of which 191 amplicons were polymorphic. Amplification products obtained per primer ranged from as low as 5 to maximum of 18, with an average of 11.55 bands per primer (**Table 2**). Percent polymorphism ranged from 76% (MOSSELLER-147) to 100% (MOSSLLER-188 and 198), with an average of 82.68%.

The genetic similarity matrix of all pair wise combination of populations is presented in **Table 3**. Genetic similarity analysis showed considerable variation among the populations. The value of similarity coefficient ranged from 0.36 to 0.78. Average Genetic distance varied from 0.23 to 0.53 between the populations. The RAPD banding patterns obtained using primer M-182 and OPA-08 is shown in **Fig. 1**.

Clustering of localities occur when the average distance between the localities is high. The dendrogram grouped the populations into five major clusters (**Fig. 2**) based on the similarity pattern. The populations of HP dominated the first cluster with the exception of one population that is from UK. This population i.e. Pokhra (UK) share 64% similarity with the populations of HP in this cluster. Similarity coefficient value (**Table 3**) between Pokhra (UK) and Hamirpur (HP) is 0.72. This cluster is recognized into two clear sub clusters I and II. The sub cluster I consisted of eight populations with an average of 64% genetic similarity among the populations. The highest similarity within this sub cluster is between Jamta (HP) and Una, Badsar (HP) and the similarity coefficient value is 0.78. Sub cluster II consisted of six populations with an average similarity of 63%. The highest similarity within this sub cluster shown in dendrogram is between Danoghat (HP) and Dharamsala (HP) and the similarity coefficient value is 0.73.

The second major cluster consisted of ten populations from the adjoining states i.e. J&K and H.P. Within this group the maximum similarity was found between the populations of Bakloh (HP) and Mastgarh (HP), Bakloh and Brindavan Nurpur (HP), and Akhnoor (JK) and Chamba (HP) with a similarity of 75% and similarity coefficient of 0.75. This group is evident to be further subdivided into two

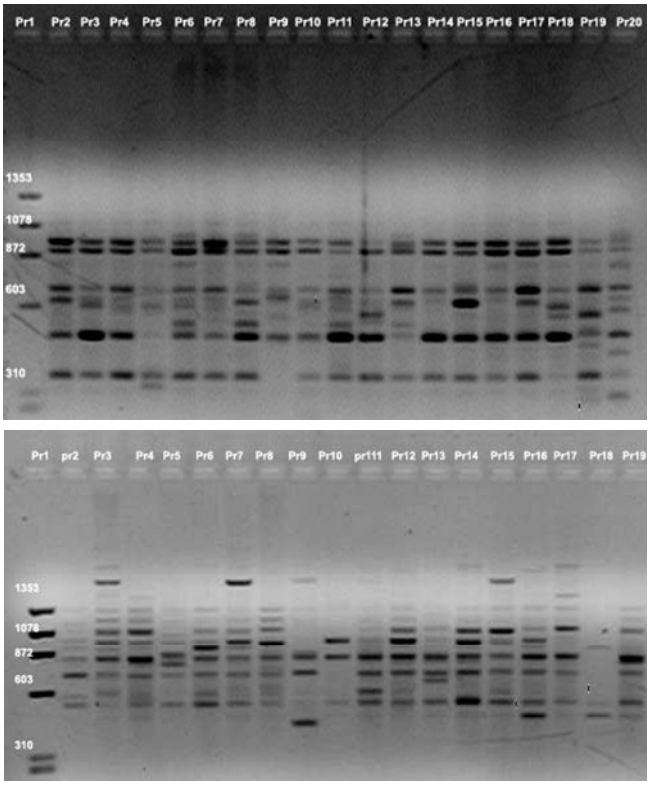

Fig. 1 RAPD profiles generated by primers OPA-08 (top) and M-182 (bottom) (P1 to P18 populations). The first lane in the each gel is ϕX-174 DNA/*Bsu*RI (*Hae*III), a molecular weight marker (sizes in bp).

sub clusters I and II. Sub clusters I consisted of populations belonging to closer geographical regions of Himachal Pradesh whereas the sub cluster II shared two populations from JK i.e., Ramnagar and Akhnoor and the rest from HP. Similarity coefficient values between Akhnoor (JK) and Bahli, Takleh (HP), Ramnagar (JK) and Palampur (HP), and Ramnagar (JK) and Taradevi (HP) are 0.71, 0.61 and 0.58, respectively.

The third cluster consisted of seven populations from the states of JK, UK, HP and Northeastern region. The highest similarity within this subcluster is between Katra (JK) and Naushera (JK) and the similarity coefficient value is

0.76. Whereas the most similar populations among HP are Kunihar (HP) and Narang (HP) with similarity coefficient value of 0.68.

Some distinguishing populations are also seen (**Fig. 2**) which are the part of three major clusters. Among these populations, Nadaun (HP) and Rajouri (JK) gave similarity coefficient value of 0.70, and Jarmola (UK) and Supin Sankri (UK) gave similarity coefficient value of 0.61.

The fourth cluster contained fourteen populations predominantly twelve from UK and two from HP. Among the populations of UK Bhowali and Ranikhet are most similar with the similarity coefficient value of 0.77, whereas the two populations from HP, Rajgarh and Sabathu showed similarity coefficient value of 0.73.

The fifth and last cluster comprises of three populations from UK state i.e. Dharmigad, Pharsula and Thadiyar. Among these Pharsula and Thadiyar are the most similar one with the similarity coefficient value of 0.69. The last population in the dendrogram i.e., Thalisian (UK) was the source that was the most dissimilar and genetically distinct and found as an outlier for the entire populations of *P. roxburghii* under study.

Standardization of RAPD conditions is an essential prerequisite for obtaining reliable amplification profiles. Our experimentation indicated the quantity and quality of DNA played a key role in determining the consistency of RAPD profiles. Besides this, the concentration of Mg ions was also found important in determining the reproducibility of RAPD profiles. From the results a DNA quantity of 5 ng and 3 mM concentration of $MgCl_2$ produced consistent and reproducible results.

Wide range of genetic distances shows that the differen-

Table 3 Genetic similarity matrix of various populations of *P. roxburghii* based on RAPD analysis.

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20	P21	P22
Akhnoor (JK)	1.00																					
Augustmuni (UK)	0.6	1.00																				
Bahli, Takleh (HP)	0.71	0.51	1.00																			
Bakloh (HP)	0.64	0.52	0.62	1.00																		
Banethi (HP)	0.68	0.59	0.62	0.55	1.00																	
Banikhet (HP)	0.63	0.47	0.70	0.71	0.62	1.00																
Barkot (UK)	0.50	0.51	0.48	0.53	0.49	0.52	1.00															
Bhowali (UK)	0.52	0.55	0.50	0.47	0.55	0.50	0.57	1.00														
Bhuntar (HP)	0.6	0.60	0.59	0.60	0.65	0.63	0.58	0.55	1.00													
Birahi (UK)	0.54	0.67	0.56	0.53	0.64	0.52	0.52	0.56	.45	1.00												
Brindavan (HP)	0.63	0.62	0.53	0.75	0.63	0.69	0.57	0.54	0.59	0.53	1.00											
Chamba (HP)	0.75	0.62	0.69	0.63	0.63	0.65	0.53	0.58	0.59	0.6	0.65	1.00										
Chiwa (UK)	0.57	0.54	0.59	0.60	0.57	0.59	0.52	0.56	0.49	0.58	0.67	0.6	1.00									
Danoghat (HP)	0.64	0.56	0.58	0.73	0.64	0.71	0.53	0.55	0.65	0.53	0.71	0.71	0.60	1.00								
Dharampur (HP)	0.52	0.49	0.50	0.62	0.67	0.60	0.45	0.48	0.69	0.46	0.56	0.51	0.42	0.62	1.00							
Dharamshala (HP)	0.64	0.59	0.58	0.64	0.59	0.67	0.49	0.62	0.70	0.53	0.71	0.71	0.60	0.73	0.67	1.00						
Dharasu (UK)	0.52	0.69	0.50	0.59	0.51	0.58	0.57	0.68	0.55	0.63	0.62	0.65	0.60	0.67	0.52	0.63	1.00					
Dharmigad (UK)	0.53	0.50	0.43	0.60	0.52	0.51	0.51	0.52	0.52	0.44	0.59	0.56	0.58	0.52	0.53	0.60	0.46	1.00				
Dhiramng (NE)	0.58	0.51	0.65	0.49	0.58	0.52	0.48	0.46	0.54	0.56	0.53	0.65	0.52	0.58	0.55	0.58	0.58	0.39	1.00			
Gangolihat (UK)	0.47	0.55	0.49	0.50	0.50	0.45	0.57	0.68	0.50	0.60	0.54	0.62	0.53	0.54	0.47	0.58	0.73	0.52	0.45	1.00		
Gaucher (UK)	0.36	0.60	0.37	0.45	0.49	0.41	0.55	0.59	0.53	0.55	0.49	0.46	0.55	0.45	0.46	0.49	0.60	0.44	0.44	0.46	1.00	
Hamirpur (HP)	0.73	0.64	0.64	0.61	0.65	0.60	0.51	0.53	0.67	0.62	0.64	0.64	0.62	0.65	0.54	0.70	0.57	0.46	0.55	0.44	0.40	1.00
Jamta (HP)	0.70	0.60	0.64	0.70	0.74	0.68	0.63	0.52	0.77	0.54	0.77	0.64	0.58	0.65	0.68	0.65	0.56	0.61	0.59	0.51	0.50	0.67
Jarmola (UK)	0.66	0.62	0.54	0.55	0.55	0.54	0.60	0.64	0.55	0.50	0.61	0.61	0.59	0.58	0.44	0.58	0.65	0.52	0.54	0.47	0.47	0.60
Jumhar (HP)	0.63	0.55	0.57	0.58	0.58	0.61	0.40	0.43	0.59	0.48	0.61	0.53	0.63	0.67	0.55	0.67	0.54	0.51	0.61	0.41	0.44	0.60
Katra (JK)	0.67	0.55	0.53	0.63	0.58	0.53	0.53	0.51	0.59	0.53	0.62	0.65	0.60	0.63	0.47	0.58	0.58	0.52	0.61	0.50	0.46	0.68
Kirala (HP)	0.64	0.63	0.63	0.55	0.72	0.58	0.54	0.59	0.70	0.57	0.59	0.63	0.61	0.60	0.62	0.60	0.63	0.45	0.58	0.51	0.50	0.69
Kothar (HP)	0.60	0.53	0.63	0.64	0.47	0.54	0.46	0.48	0.51	0.50	0.55	0.55	0.57	0.51	0.43	0.55	0.52	0.42	0.50	0.43	0.46	0.57
Kunihar (HP)	0.70	0.53	0.50	0.60	0.56	0.50	0.42	0.44	0.56	0.46	0.60	0.55	0.54	0.56	0.47	0.51	0.44	0.53	0.59	0.38	0.35	0.67
Mandi (HP)	0.60	0.52	0.49	0.50	0.55	0.49	0.41	0.51	0.60	0.49	0.46	0.54	0.45	0.59	0.62	0.68	0.51	0.44	0.62	0.46	0.42	0.61
Manikaran (HP)	0.70	0.60	0.64	0.65	0.70	0.64	0.54	0.52	0.77	0.50	0.64	0.64	0.58	0.70	0.58	0.65	0.60	0.57	0.59	0.55	0.50	0.62
Mastgar (HP)	0.71	0.48	0.69	0.75	0.58	0.69	0.45	0.47	0.59	0.49	0.65	0.65	0.60	0.58	0.60	0.54	0.55	0.52	0.57	0.46	0.42	0.60
Merwa (HP)	0.60	0.52	0.49	0.50	0.64	0.49	0.49	0.51	0.65	0.45	0.63	0.58	0.57	0.55	0.62	0.64	0.55	0.52	0.58	0.46	0.53	0.57
Mohand (UK)	0.51	0.63	0.58	0.50	0.64	0.58	0.69	0.58	0.60	0.64	0.58	0.54	0.57	0.55	0.51	0.50	0.63	0.44	0.58	0.54	0.57	0.57
Nadaun (HP)	0.58	0.50	0.51	0.67	0.57	0.60	0.60	0.57	0.58	0.51	0.65	0.61	0.55	0.67	0.65	0.62	0.57	0.50	0.56	0.48	0.55	0.59
Nafra (NE)	0.61	0.50	0.55	0.57	0.61	0.55	0.43	0.49	0.52	0.55	0.60	0.56	0.62	0.57	0.49	0.57	0.53	0.42	0.60	0.52	0.47	0.58
Nandpryag (UK)	0.56	0.63	0.44	0.56	0.59	0.47	0.58	0.62	0.52	0.54	0.62	0.52	0.57	0.59	0.49	0.52	0.62	0.50	0.51	0.55	0.63	0.54
Narang (HP)	0.67	0.58	0.51	0.57	0.62	0.51	0.47	0.49	0.58	0.55	0.61	0.57	0.63	0.57	0.49	0.57	0.53	0.50	0.47	0.48	0.47	0.68
Naushera (JK)	0.61	0.64	0.55	0.70	0.65	0.60	0.55	0.53	0.57	0.62	0.72	0.64	0.62	0.65	0.54	0.65	0.64	0.58	0.51	0.56	0.51	0.63
Palampur (HP)	0.65	0.61	0.64	0.61	0.65	0.60	0.47	0.53	0.62	0.55	0.64	0.64	0.62	0.65	0.63	0.70	0.60	0.54	0.55	0.56	0.44	0.71
Pharsula (UK)	0.62	0.58	0.49	0.62	0.62	0.57	0.63	0.63	0.63	0.56	0.61	0.64	0.52	0.65	0.51	0.62	0.58	0.66	0.49	0.54	0.49	0.63
Pokhra (UK)	0.54	0.58	0.52	0.58	0.62	0.61	0.48	0.54	0.63	0.59	0.61	0.57	0.63	0.71	0.60	0.62	0.65	0.43	0.61	0.53	0.48	0.72
Rajgarh (HP)	0.61	0.68	0.64	0.60	0.65	0.59	0.58	0.52	0.72	0.62	0.68	0.64	0.58	0.65	0.58	0.70	0.60	0.49	0.50	0.60	0.50	0.71
Rajouri (JK)	0.67	0.45	0.60	0.56	0.56	0.55	0.55	0.52	0.51	0.50	0.51	0.65	0.54	0.62	0.59	0.51	0.57	0.49	0.55	0.56	0.46	0.59
Ramnagar (JK)	0.65	0.58	0.60	0.69	0.65	0.64	0.59	0.53	0.53	0.59	0.68	0.68	0.62	0.57	0.59	0.57	0.57	0.62	0.52	0.57	0.48	0.67
Ranikhet (UK)	0.56	0.56	0.54	0.59	0.51	0.50	0.54	0.77	0.55	0.57	0.58	0.62	0.50	0.59	0.48	0.67	0.69	0.56	0.46	0.65	0.53	0.53
Sabathu (HP)	0.53	0.58	0.51	0.48	0.57	0.56	0.64	0.49	0.68	0.43	0.57	0.61	0.43	0.52	0.54	0.62	0.45	0.46	0.51	0.48	0.39	0.55
SUKet (HP)	0.64	0.56	0.62	0.50	0.64	0.58	0.49	0.65	0.65	0.49	0.54	0.71	0.53	0.64	0.62	0.73	0.59	0.52	0.67	0.54	0.45	0.65
Supin Sankri (UK)	0.59	0.62	0.54	0.63	0.47	0.50	0.46	0.52	0.55	0.60	0.58	0.62	0.6	0.59	0.43	0.63	0.59	0.56	0.50	0.58	0.47	0.60
Taradevi (HP)	0.71	0.62	0.57	0.58	0.67	0.57	0.53	0.51	0.50	0.56	0.69	0.73	0.67	0.63	0.51	0.71	0.58	0.63	0.57	0.58	0.49	0.64
Thadiyar (UK)	0.54	0.57	0.56	0.57	0.49	0.48	0.66	0.56	0.53	0.52	0.56	0.60	0.58	0.57	0.46	0.57	0.60	0.58	0.48	0.60	0.52	0.55
Thalisian (UK)	0.46	0.41	0.37	0.42	0.49	0.44	0.48	0.56	0.45	0.45	0.49	0.49	0.61	0.53	0.46	0.49	0.43	0.51	0.52	0.42	0.45	0.47
Theture (UK)	0.50	0.60	0.52	0.45	0.57	0.52	0.66	0.72	0.49	0.65	0.53	0.56	0.61	0.53	0.50	0.53	0.73	0.47	0.56	0.63	0.55	0.62
Trisula (UK)	0.58	0.68	0.53	0.50	0.58	0.53	0.6	0.63	0.58	0.62	0.57	0.57	0.59	0.58	0.43	0.58	0.54	0.45	0.45	0.54	0.59	0.67
Una, Badsar (HP)	0.67	0.65	0.65	0.67	0.67	0.65	0.55	0.49	0.74	0.55	0.70	0.61	0.63	0.71	0.65	0.71	0.65	0.54	0.65	0.48	0.51	0.68

Table 3 (Cont.)

	P23	P24	P25	P26	P27	P28	P29	P30	P31	P32	P33	P34	P35	P36	P37	P38	P39	P40	P41	P42	P43
Jamta (HP)	1.00																				
Jarmola (UK)	0.63	1.00																			
Jumhar (HP)	0.68	0.57	1.00																		
Katra (JK)	0.68	0.64	0.61	1.00																	
Kirala (HP)	0.74	0.62	0.58	0.59	1.00																
Kothar (HP)	0.52	0.52	0.58	0.59	0.56	1.00															
Kunihar (HP)	0.62	0.52	0.64	0.68	0.61	0.61	1.00														
Mandi (HP)	0.56	0.55	0.62	0.58	0.64	0.43	0.56	1.00													
Manikaran (HP)	0.76	0.63	0.64	0.72	0.70	0.48	0.67	0.65	1.00												
Mastgar (HP)	0.72	0.58	0.61	0.62	0.67	0.59	0.60	0.54	0.64	1.00											
Merwa (HP)	0.70	0.58	0.53	0.67	0.6	0.38	0.51	0.59	0.70	0.58	1.00										
Mohand (UK)	0.65	0.55	0.44	0.54	0.60	0.47	0.47	0.45	0.60	0.50	0.55	1.00									
Nadaun (HP)	0.68	0.53	0.56	0.61	0.62	0.58	0.59	0.57	0.63	0.57	0.57	0.67	1.00								
Nafra (NE)	0.62	0.56	0.68	0.72	0.57	0.57	0.58	0.61	0.58	0.64	0.65	0.48	0.55	1.00							
Nandpryag (UK)	0.64	0.58	0.55	0.59	0.56	0.46	0.57	0.48	0.64	0.48	0.48	0.63	0.69	0.5	1.00						
Narang (HP)	0.68	0.53	0.60	0.65	0.67	0.53	0.68	0.52	0.68	0.57	0.62	0.57	0.65	0.5	0.62	1.00					
Naushera (JK)	0.71	0.63	0.60	0.76	0.65	0.61	0.62	0.52	0.71	0.60	0.65	0.61	0.64	0.67	0.57	0.64	1.00				
Palampur (HP)	0.71	0.56	0.64	0.68	0.69	0.61	0.58	0.57	0.71	0.64	0.61	0.57	0.59	0.58	0.57	0.64	0.71	1.00			
Pharsula (UK)	0.55	0.54	0.49	0.57	0.51	0.47	0.55	0.46	0.55	0.50	0.54	0.54	0.52	0.48	0.55	0.52	0.59	0.56	1.00		
Pokhra (UK)	0.64	0.54	0.65	0.57	0.71	0.50	0.55	0.67	0.59	0.57	0.53	0.53	0.60	0.64	0.55	0.60	0.55	0.64	0.53	1.00	
Rajgarh (HP)	0.71	0.56	0.59	0.64	0.61	0.61	0.52	0.51	0.67	0.55	0.60	0.65	0.59	0.53	0.57	0.68	0.67	0.71	0.55	0.68	1.00
Rajouri (JK)	0.58	0.56	0.50	0.60	0.62	0.57	0.58	0.56	0.63	0.65	0.51	0.56	0.70	0.59	0.53	0.54	0.59	0.63	0.47	0.60	0.58
Ramnagar (JK)	0.71	0.63	0.48	0.68	0.65	0.58	0.54	0.45	0.63	0.68	0.53	0.57	0.64	0.55	0.58	0.60	0.67	0.67	0.56	0.52	0.58
Ranikhet (UK)	0.60	0.61	0.46	0.55	0.52	0.44	0.48	0.55	0.60	0.51	0.51	0.51	0.49	0.45	0.56	0.49	0.60	0.57	0.58	0.50	0.60
Sabathu (HP)	0.63	0.57	0.47	0.57	0.53	0.44	0.49	0.52	0.59	0.43	0.57	0.62	0.55	0.45	0.50	0.50	0.55	0.55	0.52	0.47	0.73
Suket (HP)	0.65	0.58	0.58	0.58	0.60	0.47	0.51	0.64	0.60	0.55	0.64	0.55	0.57	0.57	0.52	0.52	0.61	0.57	0.58	0.62	0.60
Supin Sankri (UK)	0.52	0.61	0.54	0.62	0.48	0.59	0.52	0.47	0.60	0.47	0.51	0.43	0.53	0.53	0.49	0.57	0.60	0.60	0.54	0.62	0.68
Taradevi (HP)	0.72	0.61	0.65	0.62	0.67	0.59	0.60	0.54	0.64	0.58	0.58	0.54	0.61	0.56	0.62	0.70	0.72	0.72	0.54	0.61	0.64
Thadiyar (UK)	0.50	0.53	0.44	0.60	0.43	0.54	0.50	0.38	0.58	0.49	0.53	0.49	0.47	0.51	0.51	0.47	0.58	0.58	0.69	0.44	0.58
Thalisian (UK)	0.46	0.47	0.44	0.56	0.43	0.39	0.54	0.49	0.46	0.39	0.53	0.57	0.63	0.55	0.57	0.47	0.51	0.47	0.52	0.52	0.42
Thetture (UK)	0.54	0.59	0.44	0.56	0.64	0.46	0.46	0.42	0.50	0.49	0.59	0.68	0.51	0.44	0.60	0.55	0.58	0.62	0.62	0.59	0.58
Trisula (UK)	0.63	0.60	0.57	0.57	0.58	0.51	0.51	0.50	0.55	0.55	0.50	0.65	0.60	0.56	0.65	0.64	0.56	0.59	0.60	0.68	0.71
Una, Badsar (HP)	0.78	0.60	0.74	0.65	0.71	0.58	0.63	0.67	0.78	0.65	0.71	0.62	0.65	0.59	0.62	0.70	0.68	0.73	0.56	0.70	0.73

Table 3 (Cont.)

	P44	P45	P46	P47	P48	P49	P50	P51	P52	P53	P54	P55
Rajouri (JK)	1.00											
Ramnagar (JK)	0.68	1.00										
Ranikhet (UK)	0.52	0.50	1.00									
Sabathu (HP)	0.43	0.55	0.49	1.00								
Suket (HP)	0.56	0.53	0.63	0.62	1.00							
Supin Sankri (UK)	0.52	0.61	0.55	0.49	0.55	1.00						
Taradevi (HP)	0.65	0.72	0.58	0.52	0.67	0.58	1.00					
Thadiyar (UK)	0.50	0.55	0.60	0.47	0.49	0.60	0.49	1.00				
Thalisian (UK)	0.46	0.45	0.40	0.51	0.53	0.43	0.49	0.48	1.00			
Thetture (UK)	0.54	0.62	0.57	0.51	0.57	0.47	0.60	0.58	0.55	1.00		
Trisula (UK)	0.47	0.49	0.58	0.60	0.54	0.61	0.57	0.49	0.52	0.56	1.00	
Una, Badsar (HP)	0.54	0.55	0.57	0.60	0.62	0.65	0.65	0.59	0.47	0.55	0.64	1.00

tiation among population is high. 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 analysis. The percent polymorphism ranged from 77 to 100% with an average of 82.68% indicates considerable variation in the species. Genetic variation in *P. roxburghii* populations could be due to the fact that this species grows over a wide range of geographic conditions in Himalaya. High genetic variation is also attributed to the high cross-pollinating nature of this species. It covers vast areas and is differentiated into many provenances and races. Each provenance is adapted to a distinct local climate, and also to altitudinal and latitudinal ranges. The genetic similarities obtained from the analysis can also be used for selection of parents to generate mapping populations and for selecting breeding populations. From the present investigation chir pine can be characterized by considerable variation and the dendrogram constructed through analysis can be used for making the seed zones in this species for tree improvement and conservation efforts. The highly divergent character of *P. roxburghii* revealed by our present study also supports molecular evidence by Liston *et al*. (1999).

Occurrence of populations predominantly of HP, JK and UK in the first and second cluster is due to close vicinity of these three states with a more or less similar terrain. Populations might have developed their genetic system through continuous gene flow with less reproductive barriers. It is also quite possible that the exchange of the genetic material between these states have resulted in those populations becoming genetically closer to each other. Gene flow through pollen grains in *P. roxburghii* is not uniform in all directions and that they can travel up to 2.5 km towards the downhill direction, while in the uphill direction dispersion is restricted to 320 m from the source (Sharma and Khanduri 2007). Pollen frequency is highest within 50-100 m periphery of the source and declined rapidly as the distance from the source increased (Sharma and Khanduri 2007). The gene flow mechanism through seeds and pollen governs the overall genetic structure and diversity in *P. roxburghii*. The populations, which are from higher elevations, have more distinctive features than at the lower elevations. However, cluster analysis reflected no clear grouping according to geographic locations of the populations. Irregular

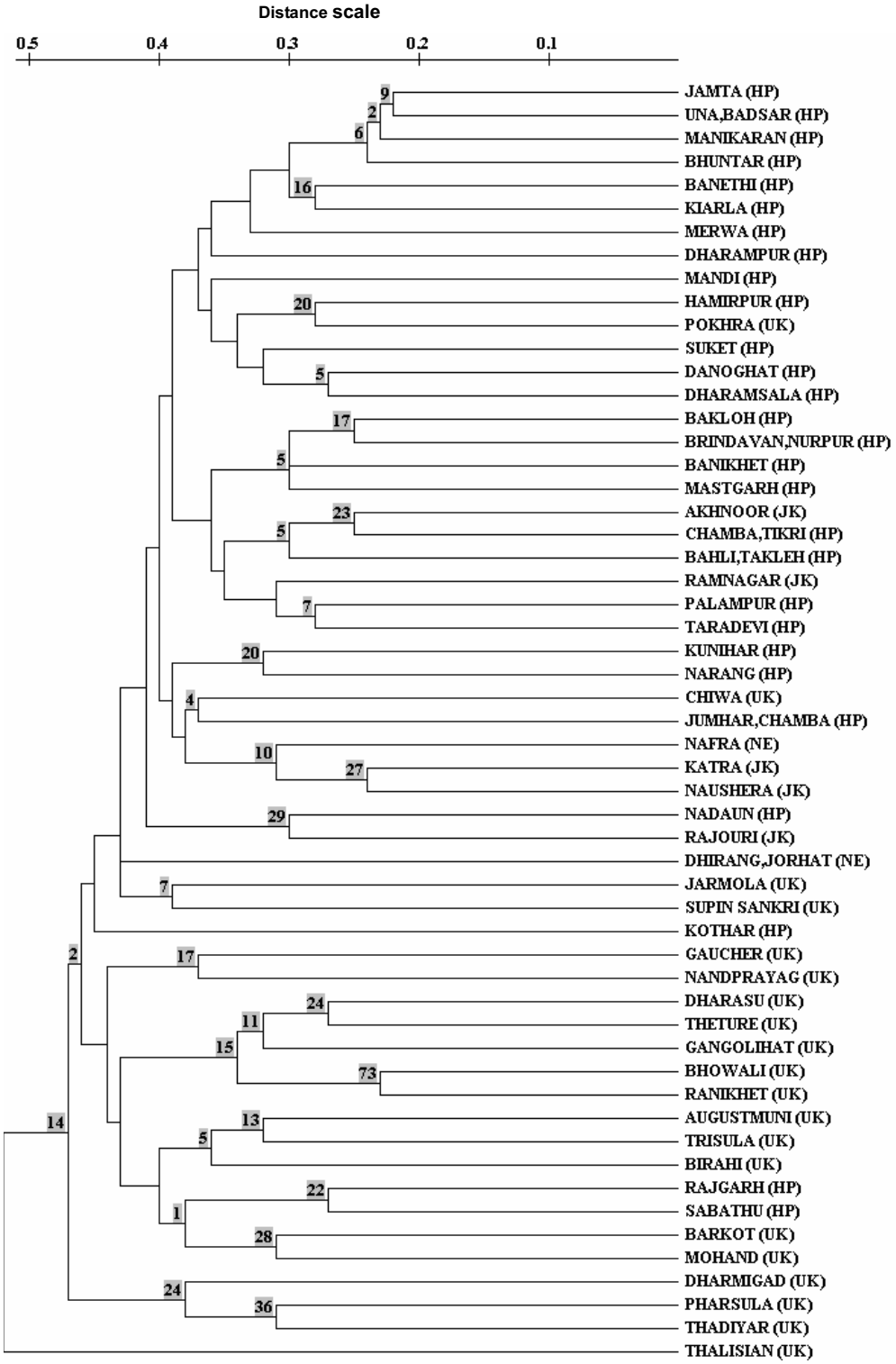

Fig. 2 Genetic divergence between 55 populations of chir pine (*P. roxburghii*), based on UPGMA cluster analysis.

changes in genetic distance between populations suggest that the variation was not clinal. The species is known to occur in distinctly different ecotypes, which has been clearly reflected in the dendrogram. High genetic variation is also attributed to the high cross-pollinating nature of this species. The existence of variation in this material has been previously reported, based on phenotypic characters by Roy (2004). Genetic variation in chir pine as apparent from the present investigation is also due to vast variation in altitude and longitude of its occurrence.

Some of the populations are geographically isolated in higher Himalayas, making those populations prone for self-evaluation and restricting their pollen movement. Based on similarity coefficients and cluster analysis, population Thalisian (UK) was found to be quite distinct. The crossability patterns as reviewed by Kedarnath (1984), it appears that strong reproductive barrier exists for this population as it occurs in higher altitudinal range. The genetic similarities obtained from the analysis can also be used for selection of parents to generate mapping populations and for selecting breeding populations. The wide genetic base revealed in the present investigation emphasizes the need to exploit the variability through selection under improvement program.

ACKNOWLEDGEMENTS

The authors thank to the Department of Biotechnology, Govt. of India for providing financial support for conducting this study.

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