

Genetic divergence in half-sib progenies of *Pinus wallichiana* A.B. Jackson plus trees in the Kashmir Himalaya, India

MOHD. ASLAM¹, ZAFAR A. RESHI^{2*} & T. O. SIDDIQI³

¹State Forest Research Institute, Srinagar 190001, J & K, India

²Department of Botany, University of Kashmir, Srinagar 190006, J & K, India

³Department of Botany, Jamia Hamdard (Hamdard University), New Delhi 110062, India

Abstract: Studies were undertaken to assess the extent of genetic divergence among the progenies of eighty-eight plus trees selected from the entire distributional range of *Pinus wallichiana* in Kashmir Himalaya at the age of one year to identify the promising selections for use in future improvement programmes. The study resolved the eighty eight plus tree progenies into ten clusters using Mahalanobis D² statistics. Cluster IV was largest with 21 genotypes (plus tree progenies). The inter-cluster distance was highest (9.901) between cluster IX and VII. Clusters mean indicated that cluster VIII was the best in respect of seedling height (12.82 cm), collar diameter (9.62 mm), needle length (5.76 cm), needle diameter (0.38 mm) and number of needles per seedling (17.44). The superiority of cluster VIII allows use of Arthnari and Seer plus tree progenies for operational plantation purposes.

Resumen: Se llevaron a cabo estudios para evaluar el grado de divergencia genética entre las progenies de ochenta y ocho árboles plus seleccionados en toda el área de distribución de *Pinus wallichiana* en los Himalaya de Cachemira, a la edad de un año, con el fin de identificar selecciones promisorias para su uso en futuros programas de mejoramiento. El estudio resolvió las ochenta y ocho progenies de los árboles plus en diez grupos por medio del estadístico Mahalanobis D². El Grupo IV fue el más grande, con 21 genotipos (progenies de los árboles plus). La mayor distancia entre grupos (9.901) se obtuvo entre los Grupos IX y VII. Las medias de los grupos indicaron que el Grupo VIII fue el mejor respecto a la altura de la plántula (12.82 cm), diámetro de la base (9.62 mm), longitud de las acículas (5.76 cm), diámetro de las acículas (0.38 mm) y número de acículas por plántula (17.44). La superioridad del Grupo VIII permite el uso de las progenies de árboles plus Arthnari y Seer para propósitos de operación de las plantaciones.

Resumo: Este estudo foi efectuado para avaliar a extensão da divergência genética entre descendências com a idade de um ano de oitenta e oito árvores plus seleccionadas no conjunto da área de distribuição do *Pinus wallichiana* na Caxemira para identificar as selecções promissoras para uso em futuros programas de melhoramento. Das descendências das oitenta e oito árvores plus, o presente estudo, usando o estatístico D² de Mahalanobis, segregou 10 agrupamentos. O sub-agrupamento IV foi o maior com 21 genótipos (descendências de árvores plus). A distância entre agrupamentos foi maior entre o agrupamento IX e VII (9,901). A média dos agrupamentos indicou que o agrupamento VIII foi a melhor em relação à altura da plântula (12,82 cm), o diâmetro do colo (9,62 mm), o comprimento da agulha (5,76 cm), diâmetro da agulha (0,38 mm) e o número de agulhas por plântula (17,44). A superioridade do agrupamento VIII permite o uso das descendências das árvores plus Arthnari e Seer para propósitos operacionais de plantação.

* Corresponding Author; e-mail: zreshi@yahoo.com

Key words: Cluster analysis, genetic variation, Kashmir Himalaya, *Pinus wallichiana*.

Introduction

Pinus wallichiana (blue pine/kail), the finest pine of north-western Himalayan region, is well known for its commercial and ecological importance (Dar 2004). The species grows naturally along the entire length of temperate Himalaya ranging in altitude from 2000 to 3,500 m above mean sea level (amsl) (Troup 1921). Of the Indian pines, the wood of the blue pine is considered to be the best and stands next to deodar (*Cedrus deodara*) in value (Aslam 2005). Though exploited mainly for timber, the species is a good source of oleoresin also, which is used for production of turpentine oil, rosin, needle oil and camphor (Verma 1988). In addition, it is a dominant species of forest ecosystem of the Kashmir Himalaya (Aslam & Salathia 2001). The species has widespread distribution in Indian Himalaya, Afghanistan, Bhutan, Pakistan and Nepal and exhibits enormous variability in its growth, form, vigour and fecundity, resin yield and quality among provenances, sites within a provenance, stands within a site, and different trees within a stand. Therefore, a significant genetic gain can be achieved by identification and selection of plus trees with desirable traits. It is an established fact that good quality progenies can also be obtained from such plus trees. Thus, precise information on the nature and degree of genetic divergence in respect of important traits is a pre-requisite for undertaking any meaningful breeding programme (Chaturvedi & Pandey 2001) for improvement and conservation of a species (Ayad *et al.* 1995; Gradual *et al.* 1999). For instance, progenies acquired after hybridizing diverse parents would exhibit broad spectrum of genetic variability and it is very likely that a segregant with valuable traits could be obtained. For these reasons, quantitative nursery seedling characteristics defining genotypes in assessing the magnitude of genetic divergence in half-sib progenies using Mahalanobis D² statistics have been successfully employed in a number of tree species including, *Pinus kesiya* (Burley & Burrows 1972), *Pinus taeda* (Owino 1977), *Eucalyptus tereticornis* (Surendran & Chandershekharan 1988), *Acacia nilotica* (Bagchi 1992), wild apricot (Singh & Chaudhary 1992), *Pinus gerardiana* (Chaudhary &

Singh 1995) and *Bombax ceiba* (Chaturvedi & Pandey 2001). However, no such study has been conducted on *P. wallichiana* in Kashmir Himalaya of Jammu and Kashmir state, India. In view of paucity of this information, present studies were carried out on progenies of 88 plus trees of *P. wallichiana* selected from 100 candidate trees.

Material and methods

The experimental material for the present study included seeds of eighty-eight pre-selected plus trees of *P. wallichiana* distributed in different forest divisions of Kashmir Himalaya (Fig.1 & Table 1). The plus trees were identified after detailed survey and inspection of all the *P. wallichiana* dominated stands in Kashmir Himalaya on ICFRE-WB funded, RGF, project no-40-5/ ICFRE (R)-97 titled, "Coordinated research project on improvement of *Pinus wallichiana* (blue pine/ kail)". The selection of plus trees is a pre-requisite for assessing the extent of genetic divergence in the existing base population of a species showing large variations, within and between populations, including blue pine, which is characterized by wind pollination coupled with wide geographical distribution (Sehgal & Sharma 2002). Hence, the selection of quality plus trees is an essential and basic step to all tree improvement and conservation strategies (Kant *et al.* 2006). In all 100 candidate plus trees along with 500 check trees from 15 sites distributed over the entire distributional range of *P. wallichiana* in Kashmir Himalaya were selected. On comparison of morphological characters (tree height, clear bole height, crown length and diameter, girth at breast height, diameter at breast height, diameter at mid point of height, bole volume, taper, straightness, branch thickness, branch angle, pruning ability, etc.) of the candidate plus trees with the mean data of five check trees, 88 plus trees (hereafter referred as families), representing elite germplasm of the species, were identified. The seeds of these plus trees, after soaking in water for six days at 3-5 °C, were sown during March 2001, in perforated polybags of 20 cm x 10 cm size with four replications having 15 polybags per replication for each plus tree. The medium used for raising the seedlings was composed of forest soil, sand and well decomposed kail humus in the ratio of 2:1:1.



Fig. 1. Map of Jammu and Kashmir State (India) showing location of different seed source sites of 88 plus trees of *Pinus wallichiana*.

Table 1. Geographic location, climatic and seed source details of *Pinus wallichiana*.

Forest division	Latitude (°N)*	Longitude (°E)*	Altitude (m)*	Annual rainfall (mm)!	Location**	Compartment no.	No. of plus trees selected
Lidder	34° 02'	75° 20'	2400	1075.2	Batakote (1)	28/L	6
Lidder					Pahalgam (2)	47/L	8
Lidder					Arthnari (3)	38/L	7
Lidder					Seer (4)	3/L	5
Lidder					Braid (5)	10/L	4
Lidder					Hapatnar (6)	12/L	7
Lidder					Liddru(7)	31/L	4
Pirpanjal	34° 05'	74° 20'	3000	2012.5	Batawooder(8)	11/D	10
Pirpanjal					Neegu (9)	18/D	10
Pirpanjal					Haijan (10)	22/D	10
Shopian	33° 50'	74° 40'	2166	768.5	Haripora (11)	5/Rb	10
Shopian					Sedow (12)	7/V	1
Shopian					Sedow (13)	9/V	1
Sindh	32° 58'	72° 30'	1660	549.4	Kangan (14)	22/S	1
Kamraj	34° 29'	76° 20'	2166	793.9	Sogam (15)	63/SL	4

Source: *Digest of Forest statistics, 2000, Forest Department (J & K Government); ! Indian Meteorological Centre, Rambagh, Srinagar, Kashmir (J & K);**Designation numbers of seed source sites are given in parenthesis.

Table 2. Analysis of variance (ANOVA) for different characteristics of half-sib progenies of plus trees in *P.wallichiana*.

Source of variation	df	MSS					
		Seedling height (cm)	Collar diameter (mm)	Needle length (cm)	Needle diameter (mm)	Needles/seedling (no.)	Survival (%)
Replication	3	0.505	0.866	0.842	0.439	0.898	1.98
Families (Genotype)	87	2.423**	1.320**	1.479**	0.698	1.786**	2.803**
Error	261	0.3811	0.147	0.5512	0.181	0.575	0.713

MSS = Mean sum of square; df = Degree of freedom; ** = Significant at 1 % level.

Table 3. Clustering among 88 plus tree progenies of *P. wallichiana*.

Cluster no.	No. of plus tree progenies/cluster	Source/Designation*
I	8	Haijan (42, 43, 44), Sogam (65, 67), Hapatnar (87), Braid (78, 79)
II	1	Haripora (54)
III	18	Batakote (2), Pahalgam (7, 11, 12), Batawooder (26, 27, 29), Neegu (35, 36, 37, 38, 39, 40, 41), Haijan (45, 46), Seer (73), Liddru (96)
IV	21	Pahalgam (8, 9), Arthnari (20, 21), Batawooder (28), Haijan (48, 49, 51), Haripora (53, 55, 56, 58, 60, 61), Sedow (62, 63), Kangan (64), Sogam (69), Hapatnar (89, 93), Seer (74)
V	10	Batawooder (25), Haijan (47), Haripora (52), Seer (75), Hapatnar (91, 92, 94, 95), Liddru (97, 99)
VI	1	Pahalgam (14)
VII	10	Arthnari (17, 19), Batawooder (22, 30), Neegu (33, 34), Haijan (50), Haripora (57, 59), Liddru (100)
VIII	4	Arthnari (15, 16, 18), Seer (72)
IX	1	Pahalgam (10)
X	14	Batakote (1, 3, 4, 5, 6), Pahalgam (13), Batawooder (23, 24, 31), Neegu (32), Sogam (66), Seer (71), Braid (77, 81).

* Designation numbers of plus trees are given in parenthesis.

Table 4. Average inter- and intra-cluster distance (D^2) in progenies of *Pinus wallichiana*.

Cluster no.	I	II	III	IV	V	VI	VII	VIII	IX	X
I	1.075									
II	8.912	0.004								
III	2.041	8.784	0.924							
IV	1.811	9.030	1.483	1.091						
V	2.288	8.918	1.547	2.361	1.073					
VI	8.392	12.576	8.107	7.538	7.398	0.001				
VII	3.025	9.079	1.757	2.580	2.079	8.336	1.419			
VIII	4.411	9.644	3.334	3.196	3.935	7.959	2.376	1.635		
IX	9.745	13.470	9.512	9.413	9.651	11.965	9.901	9.886	1.423	
X	2.711	9.150	1.479	1.895	2.398	7.925	3.129	4.273	9.366	1.101

Bold figures are intra-cluster distances.

The bags were arranged in a randomized block design and placed at the Forest Research Nursery, Seer of State Forest Research Institute, J & K, which is situated about 2.5 km from Seer village on Khanabal - Pahalgam road and 12 km from main Anantnag town. The nursery is located in compartment no.03/Lidder Forest Division at an elevation of 1,900 meters above mean sea level (amsl) on the western aspect and lies at 34° 17' N latitude 75° 20' E longitude. Cultural operations with regard to watering and weeding were kept uniform in the entire experimental set up.

After one year from the date of sowing, 5 seedlings per replication, selected at random, were tagged with aluminium tags for recording data on the growth parameters viz., seedling height (cm), collar diameter (mm), needle length (cm), needle diameter (mm), needles/seedling (no.) and survival percentage (%). Seedling height was measured from medium surface to the tip of the seedling with the help of a centimeter scale. Collar and needle diameters were measured using digital vernier caliper and needle length was recorded with a centimeter scale. Number of needles per seedling is based on mean values of needles of five seedlings. Survival percentage was calculated by dividing the number of seedlings of a family surviving in each replication by actual number of seedlings raised for that family and then multiplied by 100. Using mean values all of the above mentioned traits of the one year seedlings, data were subjected to analysis of variance (ANOVA) to decompose the variability into genetic and environmental components (Gardner 1963). The data collected were then analyzed through Mahalanobis D^2 statistics (Chaturvedi & Pandey 2001) for estimating the genetic divergence among the one-year old progenies of 88 plus trees. The concept of Mahalanobis genetic distances, among the multivariate distance methods, has been shown to be robust (Dias & Kageyama 1997) because of having vital utility in differentiating well defined populations, which is an approach basic and fundamental for estimating the extent of diversity among the selected genotypes (Kole & Saha 2009). The more diverse the parents within the overall limit of fitness, the greater the chances of obtaining a higher amount of heterotic expression and a broad spectrum of variability in segregation generations (Kole & Mishra 2002). Owing to large amount of genetic variations encountered in wind pollinated conifers, it becomes an essential requirement to assess and analyze the extent of this genetic divergence in the germplasm of a species

through the use of this scientific and advanced biometrical technique viz., multivariate technique based on Mahalanobis D^2 statistics. The clusters, derived from distance matrices, were formed according to Tocher's algorithm (see Rao 1952). This procedure starts with two closely associated genotypes (represented here by plus trees) and finds a third genotype which has the smallest average D^2 from the first two. Next, the fourth is chosen to have smallest average D^2 from the first three, and so on. There is an increase in the average D^2 within a cluster whenever an additional genotype is included. The limit for inclusion of a new genotype corresponds to the minimum generalized distance, above which the newly added genotype has to be considered as outside the former cluster.

The intra-cluster distances were worked out by rating the average of the component progenies in that cluster whereas the average inter-cluster divergences were calculated by taking into consideration all the component D^2 values possible among the members of two clusters considered. The square root of the average D^2 values gives the genetic distances between the progenies.

Results

In the present study, a total of six characters were considered and the significant differences were observed among the progenies indicating that a considerable amount of genetic variability existed for all these characters except for the needle diameter (Table 2).

Application of Mahalanobis statistics and Tocher's technique allowed grouping of the 88 plus tree progenies into 10 distinct clusters on the basis of Mahalanobis D^2 statistic (Table 3), indicating adequate genetic variability among the clusters under study. Maximum number of genotypes (21) was grouped in cluster IV, followed by cluster III (18) and cluster X (14). Clusters V and VII consisted of 10 genotypes each followed by clusters I and VIII which included 8 and 4 genotypes, respectively. Cluster III, VI and IX were distinct and unique from others as each included only one genotype indicating their uniqueness from the breeding point of view. On the contrary, clusters III, IV, VIII and X included selections from all the locations.

The average intra - and inter - cluster distances (Table 4) indicate that intra-cluster distances range from 0.001 to 1.635. The highest intra-cluster value (1.635) was obtained for cluster VIII followed by cluster IX (1.423). The inter-cluster

Table 5. Cluster means of 88 plus tree progenies with respect to different traits in *P. wallichiana*.

Cluster no.	Seedling height (cm)	Collar diameter (mm)	Needle length (cm)	Needle diameter (mm)	Needles per seedling (no.)	Survival (%)
I	11.41	6.65	4.39	0.32	16.38	50.94
II	9.90	5.57	4.80	0.29	16.50	70.25
III	12.11	7.14	4.76	0.31	15.42	80.12
IV	11.89	7.82	5.35	0.32	15.32	60.70
V	11.25	6.24	5.85	0.33	16.42	80.25
VI	9.07	7.55	4.68	0.32	15.50	60.75
VII	11.90	7.65	4.80	0.31	17.38	90.20
VIII	12.82	9.62	5.76	0.38	17.44	90.00
IX	10.36	7.53	5.50	0.28	13.00	70.50
X	11.52	6.99	5.11	0.30	13.45	70.84

distances varied from 1.479 to 9.901. The lowest inter - cluster distance of 1.479 was observed between cluster X and III and the highest inter-cluster distance of 9.901 was observed between cluster IX and VII, indicating a high degree of genetic divergence among the plus tree progenies. Mean performance of divergent clusters reveal (Table 5) that cluster VIII has the highest mean value for seedling height (12.82 cm), collar diameter (9.62 mm), needle length (5.76 cm), needle diameter (0.38 mm) and number of needles per seedling (7.44). This is followed by clusters III and IV in which highest mean value for height (12.11 cm) and collar diameter (7.82 mm) were recorded. In respect of seedling survival, clusters VII and VIII recorded highest values of 90.2 % and 90.0 %, respectively. Lowest percentage of seedling survival (50.94) was recorded in cluster I.

Discussion

Assessment of genetic divergence in the populations of a species is of paramount significance in purposeful breeding programmes. In fact, an appraisal of the degree of divergence in important traits of a species is of added advantage in this regard, as inter-mating of divergent groups would increase variability and help produce genetically superior planting material required for use in afforestation and reforestation programmes (Chaturvedi & Pandey 2001). Pursuant to urgent need for production of quality material required for planting in deforested areas, evaluation of entire germplasm in Kashmir Himalaya was essential so as to unravel the extent of genetic divergence in *P.*

wallichiana. To meet this requirement 88 plus trees were identified on the basis of several morphological characteristics from amongst 100 candidate plus and 500 check trees. Analysis of variance of these characters showed highly significant values ($P < 0.01$), indicating the presence of large range of variability among the selected 88 accessions. Some clusters included genotypes (represented by plus trees) from all the locations, while others included only a particularly unique and distinct genotype. Sagwal (1982), Khosla *et al.* (1994), Sehgal *et al.* (1994) and Arunprasad *et al.* (2003), while reporting similar results, have suggested that all the genotypes from a given area may not necessarily form a single cluster. Thus, the pattern of divergence is not dependent on the geographical nearness of the genotypes and such a pattern could be attributed to differences in the genetic make up of the otherwise co-occurring genotypes (Bhaumik *et al.* 1971; Chauhan & Sehgal 2001; Singh & Gupta 1968). Occurrence of genotypes from geographically different areas in the same cluster, as was evident during the present study as well, has been attributed by Kumaran (1991) to the involvement of factors that might be responsible for their genetic uniformity. Perusal of intra- and inter-cluster distances (Table 4) reveals highest inter-cluster distance of 9.01 between cluster IX and VII and parents if drawn from such genetically diverse clusters could be of particular significance in production of high heterotic effect during hybridization (Arunachalam *et al.* 1980; Singh & Singh 1981). In respect of different seedling characters considered in the

present study, the clusters did vary (Table 4) and such variations in cluster means have also been reported by Kumaran (1997) and Bagchi (2000). Surendran & Chandrasekharan (1988) studied the genetic divergence among half-sib progenies of 35 single tree selections of *Eucalyptus tereticornis* from different agro-climatic zones of Tamil Nadu at 24-month growth phase and identified promising clusters for their use in the silvicultural management of the species. Kant *et al.* (2006) separated ten populations of *Pinus gerardiana* into three similar groups on the basis of multivariate D^2 statistics and suggested that this technique can be used in the improvement of economically important traits in the species. The findings of these authors are also in line with the results of Burley & Burrows (1972), who employed multivariate analysis in *Pinus kesiya* and suggested that, the technique helps in separating a large number of plus tree progenies into many clusters and accordingly the use of superior clusters in its afforestation and reforestation programmes. As we know the picture today is that the demand for forest products is increasing as rapidly as the forests are vanishing. Hopefully such knowledge will aid propagators, geneticists and tree improvement specialists in enhancing the quality and productivity of the forest ecosystems in meeting the pressing market demand. Notwithstanding the differences, the present study clearly establishes the superiority of cluster VIII (Arthnari 17, 19 and Seer 72) under Lidder forest division and recommends propagation of material from the trees belonging to this cluster for utilization in immediate plantation programmes for better productivity of the species in the Kashmir Himalaya. The present study has further confirmed the suitability of using seedling characteristics in defining genotypes and measuring genetic distance between and within the populations.

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