

VARIABILITY ASSESSMENT OF SOME MORPHOLOGICAL TRAITS AMONG BLUE PINE (*PINUS WALLICHIANA*) COMMUNITIES IN HINDUKUSH RANGES OF SWAT, PAKISTAN

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Abstract

Pinus wallichiana dominated forest communities, located in the Hindukush ranges of Swat, Pakistan were analysed for variability in the cone and seed traits. The results disclosed significant variability in mean values, critical difference, coefficient of variation, broad sense heritability, genetic gain and genetic advance. Genotypic variance (V_g) and genotypic coefficient of variance (GCV) were noted to be lower than the corresponding environmental variance (V_e) and environmental coefficient of variability (ECV) for most of the parameters which clearly shows that these traits are under the control of environment. Both Number of male clusters/branch and 100 seeds weight are heritable traits as having higher genotypic variance and genotypic coefficient of variance. Traits like 100 seeds weight, Number of male cluster/branch, number of female cones/tree, female cone weight and number of sterile scales/cone showed moderate to high percentage of heritability indicates that these traits are under strong genetic control. These heritable additive genetic traits can be used for future breeding and tree improvement plans for the species. It is further concluded that the alleged traits should be given priority while selecting superior genotypes.

Key words: Variability, Genotypic variance (V_g), Environmental variance (V_e), Heritability (H), Genetic advance (GA), Genetic gain (GG).

Introduction

Pinus wallichiana A.B. Jackson (Blue Pine) is one of the greatest commercially important and commonly distributed pine species in the Hindukush Himalayas, extending diagonally from Afghanistan through Pakistan, India, Tibet, Nepal and Bhutan to Burma. On the globe, the species is distributed in longitudinal range between 68°E to 100°E, latitudinal range between 25°N to 36°N and the altitudinal range from between 1500 to 3800 m (Little & Critchfield, 1969, Khan, 1986). *Pinus wallichiana* prefer low temperature and may occur at high altitudes in low rainfall areas and at low altitudes (less than 2,800 m) in high rainfall areas (Khosla & Raina, 1995) in pure or mixed forests and known as *Kail* in timber trade (Sahni, 1990, Singh & Yadav, 2000). Beside inevitable source of timber wood, the species is also subjugated for high quality of resin and fuel wood. It is honored among other five needle Pines due to its resistance to blister white rust caused by *Cronartium ribicola*. *Pinus wallichiana* is the first choice as a timber and fuel wood source in the area due to its easy handiness and limited availability of *Cedrus deodara* (first choice wood in area) in most of the forests of Swat.

Genetic structure of diverse *Pinus wallichiana* populations may show variations within and among the provenance types due to varying geographic, climatic and edaphic conditions and its long evolutionary history (Rawat & Bakhshi, 2011). Provenance (seed source) variation studies in tree improvement programs are in common (Callahan, 1964, Wright, 1976, Suri, 1984). Such studies were carried out to obtain best material for good productivity (Shiv Kumar & Banerjee, 1986) and also for analysing and comparing superior and inferior characters. Analysing characters have a great importance in breeding and tree improvement programs, besides

preserving these variations intact for future research programmes (Devagiri, 1997).

Provenance variations studies based on morphological, anatomical, physiological and biochemical traits are essential for tree improvement program and are undertaken in Nepal, India, Bhutan and Pakistan (Hattermer, 1991; Thapliyal & Dhiman, 1997; Kundu & Tigerstedt, 1999; Mukherjee, 2005). In addition, cone, seed and seedling characteristics are documented for seed source variation in a number of tree species (Salazar, 1989; Isik, 1986; Dvorak *et al.*, 1996; Singh *et al.*, 1996; Thapliyal & Dhiman, 1997; Roy *et al.*, 2004; Mukherjee, 2005; Sivacioglu & Ayan, 2010).

Beside this exploitation of the tree species for timber and fuel wood results in erosion of gene pool, so it is important to evaluate the amount of variability in the prevailing germplasm of the species in Hindukush ranges of Swat. It is crucial pre-requisite for genetic improvement in the anemophilous conifers, including blue pine. As the propagative traits for a tree are most conserved and least affected by environment and are neglected during selecting provenance types. Cones and seed characters can be used as indicators for selecting superior seed source, since there are a lot of variations attributed to genetic causes. Use of physical markers was a crucial step in the early period of tree breeding, when molecular markers were not easy to detect. Many workers highlighted the importance of such studies in plant breeding (El-Kassaby, 1995; Griffin, 1982; Shea, 1987; Lee *et al.*, 1998) and gene conservation programmes (Kang *et al.*, 2003; Bilir *et al.*, 2005). Some others workers studied variations in reproductive attributes of various *Pine* species like *P. nigra* (Matziris, 1993), *P. halepensis* (Boulli *et al.*, 2001) *P. coulteri*, *P. sabiniana*, *P. torreyana* (Johnson *et al.*, 2003) and *P. brutia* (Dangasuk & Panetsos, 2004; Panetsos, 1981). Recently some workers from India also reported variations in

reproductive traits of *Pinus wallichiana* from Himachal Pradesh and Uttaranchal (Rawat & Bakhshi, 2011) and the Kashmir Himalaya (Aslam *et al.*, 2010). However, no such work is reported from Hindukush ranges of Swat Pakistan on *Pinus wallichiana* populations which is indispensable for bringing genetic improvement in the species. This study can help in production of genetically superior seeds which can be used in developing new forests and restoring existing forests in country.

The present investigations were therefore, undertaken which can assist in the initial estimation of criteria for selection of some noticeable characters and could be used as a key for the evaluation of seed source or progeny trials in *Pinus wallichiana* for further improvement. In the present study we aim 1) to analyse the variations in the reproductive traits on morphological investigations, 2) to measure the extent of variation in cones and seeds features and the extent of genetic control in these parameters, 3) to evaluate the variations within and among the populations and 4) to investigate variations and inheritance pattern in cones and seeds characters.

Material and Methods

The present study was conducted in three *Pinus wallichiana* dominated communities, located at different altitudes in Swat Hindukush ranges of Pakistan (Fig. 1).

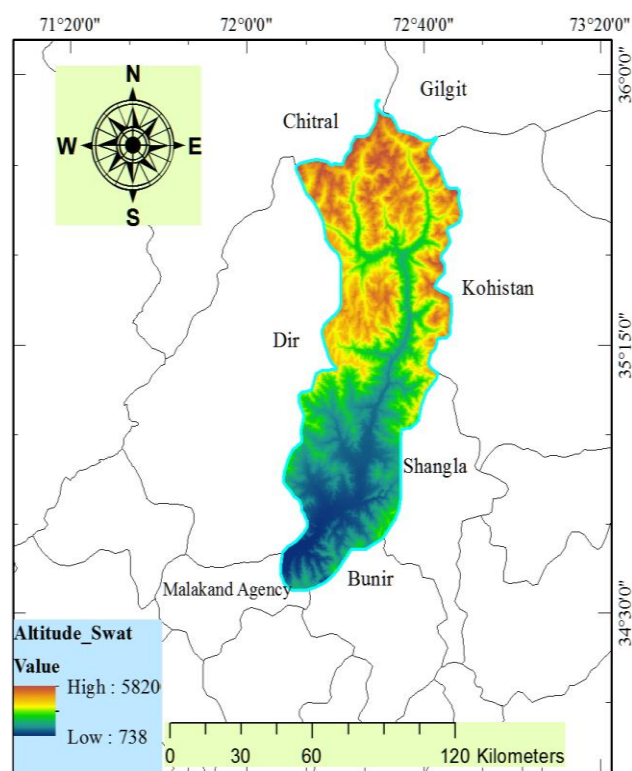


Fig. 0. Study area map showing altitudinal variation, developed with ArcGIS.

To study the variations in reproductive capacity of *Pinus wallichiana*, thirty trees were selected within each population according to FAO & Turnbull (1975). Selected trees were investigated both with a naked eye and binocular telescope for different reproductive traits *i.e.*

number of male clusters per branch (MCL), number of male cones per cluster (MCC) and number of female cones per tree (NFC).

Cone and seed characteristics: To investigate the difference in cone and seed characters, 40 undamaged mature cones from each of the three community type were collected at physiological maturity during the months of Sep-Oct (when the cones turn reddish brown). These cones were measured for size (length and diameter), dry weight, fertile scales number and sterile scales number. Seeds were expelled from dried cones by shaking mechanically. Digital balance was used to weight total seed from single cone. These seeds were then counted, de-winged, cleaned and weighted again. 100 seed weight was obtained in three replications for each community type.

Numerical investigation: Values obtained for different traits were passed through one-way analysis of variance (ANOVA) for any significant variance among the various traits or characteristics of cone in *Pinus wallichiana* populations. The critical difference was calculated at 5% level of significance. Relationships between ten seed and cone traits were tested using Pearson correlation coefficient. The variance component *i.e.*, V_p (phenotypic variance), V_g (genotypic variance), V_e (environment variance), Phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV), environment coefficient of variance (ECV), broad sense heritability (H), genetic advance (GA) and genetic gain (GG) were determined following Johnson *et al.* (1995) Burton (1952) and Burton & Devane (1953) respectively.

Results and Discussion

Analysis of variance: The results of ANOVA conclusively established significant variations among the three different *P. wallichiana* communities at 0.05 probability level for all the reproductive physiognomies examined (Table 1). The highest F value was recorded for No. of male clusters per branch (121.62) followed by number of sterile scales per cone (40.26).

Considerable variations were also observed in cone length, cone weight and number of seeds per cone. Seed size variation was observed within populations (Simons & Johnston, 2000) and larger seeds tend to have greater survival and improved performance under a wide range of environmental conditions (Manning *et al.*, 2009). Singh & Thapliyal (2012) also reported variations among *Pinus wallichiana* populations in relation to habitat. Variations in seed and cone traits are also reported in other Pine species like *P. halepensis* (Matziris, 1998) and *P. sylvestris* (Keles, 2007; Sivacioğlu & Ayan, 2010). Some other workers revealed variations in cone dimension for *P. contorta* (Owens *et al.*, 2005), in filled seed number/cone for *Pinus banksiana* and *Pinus taeda* (Todhunter & Polk, 1981; Schmidtling, 1983), in seed weight for *Picea sitchensis* (Chaisurisri *et al.*, 1992). Wang *et al.* (2014) examined intra and inter-specific variation in seed mass and morphology in relation to altitude, habitat, plant height, and phylogeny among forty-two species of Rhododendron on the Tibetan plateau.

Table 1. ANOVA for different cone and seed characters of *Pinus wallichiana* populations in Swat.

	Source of variation	SS	df	MS	F	P-value	F crit
No of male clusters per branch	Between groups	126236.6	2	63118.3	121.62	6.3E-26	3.1013
	Within groups	45149.5	87	518.960			
	Total	171386.1	89				
No of male cones per cluster	Between groups	312.0889	2	156.044	19.933	7.47E-08	3.1013
	Within groups	681.0667	87	7.82835			
	Total	993.1556	89				
No of female cones per tree	Between groups	3377.689	2	1688.84	23.743	5.91E-09	3.1013
	Within groups	6188.267	87	71.1295			
	Total	9565.956	89				
Cone length	Between groups	643.5542	2	321.777	34.184	2.04E-12	3.0738
	Within groups	1101.344	117	9.41319			
	Total	1744.898	119				
Cone diameter	Between groups	28.76491	2	14.3824	22.038	7.54E-09	3.0738
	Within groups	76.35537	117	0.65261			
	Total	105.1203	119				
Cone weight	Between groups	5426.217	2	2713.11	28.301	9.44E-11	3.0738
	Within groups	11216.38	117	95.8665			
	Total	16642.59	119				
No of fertile scales per cone	Between groups	6235.817	2	3117.91	26.839	2.55E-10	3.0738
	Within groups	13591.78	117	116.169			
	Total	19827.59	119				
No of sterile scales per cone	Between groups	958.85	2	479.425	40.265	4.95E-14	3.0738
	Within groups	1393.075	117	11.9066			
	Total	2351.925	119				
No of seeds per cone	Between groups	6204.765	2	3102.38	27.943	1.2E-10	3.0738
	Within groups	12990.04	117	111.026			
	Total	19194.8	119				
100 seed weight	Between groups	8.034756	2	4.01738	23.113	0.001516	5.1433
	Within groups	1.042867	6	0.17381			
	Total	9.077622	8				

Table 2. Appraisals of three variance components for cone and seed characters in *Pinus wallichiana* forest population of Swat, Pakistan. Check the red part?

Traits	Mean	phenotypic variance	Genotypic variance	Environmental variance	% age genotypic variance
No. of male clusters per branch	118.57	1921.59	1402.63	518.96	72.99
No. of male cones per cluster	11.82	11.30	3.47	7.83	30.70
No. of female cones per tree	27.98	108.66	37.53	71.13	34.54
Female cone length	19.82	14.78	5.36	9.41	36.29
Female cone diameter	6.55	0.89	0.24	0.65	26.86
Female cone weight	41.61	141.08	45.22	95.87	32.05
No. of fertile scales per cone	59.44	168.13	51.97	116.17	30.91
No. of sterile scales per cone	14.48	19.90	7.99	11.91	40.16
No. of Seeds per cone	56.30	162.73	51.71	111.03	31.77
100 seeds weight	4.58	1.07	0.89	0.17	83.70

Aslam *et al.* (2010) observed significant variations in cone and seed traits of *Pinus wallichiana* plus trees. Substantial differences were also reported by Rawat & Bakhshi (2011) for morphological reproductive traits indicating the genetic variability among 20 provenances of *P. wallichiana*. Thus our present results are an acceptance with the findings reported by the workers for the *P. wallichiana* and other species.

Variance components: Phenotypic, genotypic and environmental variance components of ten cone and seed related traits are given in Table 2. Traits like number of male clusters/branch, number of fertile scales/cone, number of seeds/cone and female cone

weight showed larger variance estimates than other traits. Genotypic variance in cone traits ranged from 51.97 for fertile scales/cone to 0.24 for female cone diameter. Number of male clusters/branch and 100 seeds weight showed larger genotypic variance than environmental variance. Genotypic variance accounted 73% and 84% of the total phenotypic variance for the above mentioned traits indicating high heritability in these traits. Number of sterile scales is also moderately heritable having 40% of genotypic variance. The remaining 6 cone traits reveal smaller genotypic variance than environmental variance including high value of environmental variance for the number of seeds/cone and number of fertile scales/cone.

Table 3. Values of variability, heritability, genetic advance and genetic gain for studied characters.

Traits	Phenotypic CV %	Genotypic CV %	Genetic advance (GA)	Genetic gain (GG)	Broad sense Heritability (H)
Number of male clusters per branch	1620.68	1182.99	65.91	55.59	0.73
Number of male cones per cluster	95.55	29.33	2.13	17.98	0.31
Number of female cones per tree	388.38	134.14	7.42	26.51	0.35
Female cone length	74.55	27.06	2.87	14.50	0.36
Female cone diameter	13.62	3.66	0.52	7.98	0.27
Female cone weight	339.08	108.68	7.84	18.85	0.32
No of fertile scales per cone	282.86	87.42	8.26	13.89	0.31
Number of sterile scales per cone	137.46	55.20	3.69	25.49	0.40
Number of seeds per cone	289.04	91.84	8.35	14.83	0.32
100 seeds weight	23.26	19.47	1.78	38.84	0.84

Aslam *et al.* (2010) observed similar variances in seed and cone traits in *Pinus wallichiana* at Kashmir Himalayas. Lester (1969) observed equal variation in cone size between location, trees within location and cones within a tree. These variations generally reveal a combined control of genotype and environment (local density, position in crown, climatic and edaphic conditions). High quality seeds and higher yield can be obtained from large cones having higher weight (Maheshwari & Konar, 1971). In fact, edaphic and climatic factors of an area are the most influential factors for seed characters. Different genetic architectures constructed by the diverse environmental conditions results in variations of cone and seed parameters throughout Hindukush ranges of Swat.

Variability estimates: Table 3 shows the estimates of variability, heritability, genetic advance and genetic gain for reproductive characters of *P. wallichiana* communities. Results show that Phenotypic variance and CV was greater than Genotypic variance and CV. Maximum values for all estimates (PCV, GCV, GA, GG and H) was observed in No. of male clusters/branch. 100 seeds weight showed high value for heritability (0.84) followed by number of male clusters/branch (0.73).

High value of Genetic gain (55.59) and Genetic advance (65.91) was observed for No. of male clusters/branch followed by 100 seeds weight for Genetic gain with a value of 38.84 respectively. On the other hand, values of GA, GG and heritability (H) were minimum for female cone diameter. High heritability along with high genetic advance is regarded as the true index of effective selection (Cotterill & Dean, 1990; Johnson *et al.*, 1995) as GA is a product of both the heritability and the selection intensity, or selection differential. Predicted estimates are an accomplice with the studies of Aslam *et al.* (2010), reporting High PCV than GCV as well as maximum values of Heritability, GG and GA for Cone length, cone width and 1000 seeds weight. Phenotypic expression of traits is contributed strongly by genotype while environmental factors have small effect (Khalil, 1974). Long term survival and sustainable production of any plant population depend on genetic suitability to its environment. Natural selection works on genotypes and produce populations that are physiologically attuned to a specific range of environmental conditions (Rawat & Bakshi, 2011).

Despite good values of heritability, traits (100 seeds weight, number of fertile scales/cone) did not display expected genetic gain which is possibly due to more non-

additive genetic effects than additive genetic effects. For determining proportion of heritable variations during selection of desired traits the total phenotypic variance (V_p) is partitioned into heritable (V_g) and non-heritable (V_e) components (Rawat & Bakshi, 2011). Heritability estimation is worthwhile as indicator for selection of one or more traits (Namkoong *et al.*, 1966). High percentage of heritability with high genetic gain values exhibited by 100 seeds weight and number of male clusters/branch suggested their high genetic origin with good extent of heritable additive genetic component and can be used as criterion for the selection of best progeny in this species for its improvement. Moderately heritable characters included, number of sterile scales/cone, number of female cones per tree, female cone length and cone weight, number of seeds per cone, number of male cones/cluster and number of fertile scales can also be used as selection tool. Cone diameter, cone weight, seed per cone, laboratory and nursery germination percent, germination value and seed weight are moderate to high heritable traits (Rawat & Bakshi, 2011) and are suggested to be more adequate and accurate for selecting best individuals from best seed sources (Johnson *et al.*, 1995). Findings of this study also confirm the results of Hooda & Bhadur (1993) in *Leucaena leucocephala*, Bhardwaj *et al.* (1983) in *Dioscorea deltoidea* and Mukherjee (2005) in *Pinus roxburghii*.

Correlation analysis: Pearson correlation coefficients between all the pairs of the traits in all the three communities are presented in Tables 4a, b and c respectively. Data tables reveals that female cone length showed strong to moderate positive correlation with cone weight, cone diameter, number of fertile scales and 100 seeds weight in all of the three communities. The number of fertile scales was strongly correlated with the number of seeds per cone (*Pinus-Quercus* community =0.72, pure *Pinus* community =0.96, *Pinus-Cedrus* community =0.87). Female cone weight exhibited moderate to low (*Pinus-Cedrus* community =0.45, pure *Pinus* community =0.21, *Pinus-Cedrus* community =0.27) correlation with number of fertile scales. Cone diameter also showed correlation with cone weight in the three communities with values of 0.45, 0.38 and 0.58 for *Pinus-Quercus* community, pure *Pinus* community and *Pinus-Cedrus* community respectively. In addition, the number of seeds per cone showed positive linear relationship with both number of male cones per cluster and female cone length in *Pinus-Cedrus* community. Tables 4a, b and c also shows the positive correlation the number of seeds/cone and female cone length.

Table 4a. Correlation coefficient between cones and seed characters of *Pinus wallichiana* in *Pinus-Quercus* community.

Pinus-Quercus community	MCB	MCC	FCT	FCL	FCD	FCW	FSC	SSC	SPC	100 SW
MCB	1									
MCC	0.13	1								
FCT	0.04	0.06	1							
FCL	0.13	-0.04	-0.36	1						
FCD	0.11	0.02	-0.30	0.46	1					
FCW	0.01	0.00	-0.26	0.70	0.45	1				
FSC	0.37	-0.15	-0.39	0.47	0.49	0.45	1			
SSC	-0.28	-0.12	0.27	-0.08	0.08	-0.11	-0.38	1		
SPC	0.22	-0.28	-0.25	0.37	0.36	0.30	0.72	-0.32	1	
100 SW	0.69	0.94	0.29	0.63	-0.76	0.93	-0.86	0.82	-0.93	1

Table 4b. Correlation coefficient between cones and seed characters of *Pinus wallichiana* in Pure *Pinus* community.

Pure Pinus community	MCB	MCC	FCT	FCL	FCD	FCW	FSC	SSC	SPC	100 SW
MCB	1									
MCC	-0.08	1								
FCT	0.32	0.02	1							
FCL	0.01	0.37	0.29	1						
FCD	0.22	0.04	0.27	0.36	1					
FCW	0.06	0.22	0.23	0.79	0.38	1				
FSC	-0.10	0.18	0.07	0.32	0.03	0.21	1			
SSC	0.46	-0.15	0.20	-0.19	0.02	-0.18	-0.24	1		
SPC	-0.13	0.18	0.05	0.35	0.05	0.21	0.96	-0.32	1	
100 SW	-1.00	-0.45	1.00	0.97	0.82	1.00	0.25	-0.89	0.23	1

Table 4c. Correlation coefficient between cones and seed characters of *Pinus wallichiana* in *Pinus-Cedrus* community.

Pinus-Cedrus community	MCB	MCC	FCT	FCL	FCD	FCW	FSC	SSC	SPC	100 SW
MCB	1									
MCC	0.37	1								
FCT	0.38	0.23	1							
FCL	0.30	0.14	0.35	1						
FCD	-0.04	-0.18	0.42	0.57	1					
FCW	0.22	0.00	0.33	0.65	0.58	1				
FSC	0.27	0.20	0.13	0.40	-0.03	0.27	1			
SSC	-0.09	-0.20	0.37	0.17	0.39	0.17	-0.07	1		
SPC	0.29	0.42	0.20	0.45	0.02	0.32	0.87	-0.07	1	
100 SW	0.25	-0.07	-0.66	-0.66	-0.84	-0.59	-1.00	0.84	-0.66	1

Key: MCB=No of male clusters per branch, MCC=No of male cones per cluster, FCT=No of female cones per tree, FCL = Female cone length, FCD= Female cone diameter, FCW = Female cone weight, FSC = No of fertile scales per cone, SSC = No of sterile scales per cone, SPC= No of Seeds per cone, 100 SW =100 Seeds weight

Similarly, significantly positive relationship ($r=0.54$) was observed between CDW- 1000SW by Sivacioglu (2010) and between cone weight-seed weight by Shen & Lindgren (1981) in clones of *P. sylvestris*. Ying & Iilingworth (1985) reported positive significant relationship among seed and cone characters (seed weight, cone width cone length and cone dry weight) in *P. contorta*. On the other hand, Rawat & Bakshi (2011) found significant correlation between morphological traits of cones; of which cone length was correlated with seed weight (0.723) and number of seed per cone with scales/cone (0.937). Salazar (1989), Sneizko & Stewart (1989), Bagchi & Dobriyal (1990) and Baker (1972) found relationships and reported that these traits are independent and genetically controlled. Significant correlation was also reported by Shiv Kumar & Banerjee (1986) among seed weight, speed and uniformity of germination in *Acacia nilotica* and by Mukherjee (2005)

in seed length and weight with germination percentage and germination value in *P. roxburghii*.

Conclusions

Selection is one of the important tools in breeding programs, which is based on morphogenetic behaviour of the species. Variability among members of species provides basis for selecting genetically improved plants. Seed and cones variability study within *P. wallichiana* communities of Swat provided us opportunity for selecting genetically advance seed source which can be used for further afforestation programs.

Significant variations in cone and seed traits along with genetic variability in terms of variance, coefficient of variability, broad sense heritability, genetic advance and genetic gain among different communities of *P. wallichiana* offer us an opportunity to screen out the desired characters.

Some of the seed cone characters (100 seed weight, Male clusters per branch) were highly heritable. The good heritability value along with GG and GA indicated that production and quality of seed of blue pine could be improved by simple selection of these characters. Therefore, attention must be paid to these characters while selecting seed source or establishing new plantations.

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