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ORIGINAL PAPER

Variation in cone and seed characters in blue pine (*Pinus wallichiana*) across natural distribution in western Himalayas

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Abstract: We analyzed 17 seed sources (seed stands) of *Pinus wallichiana* for variations present in cone and seed characters, scattered over natural distribution in north-west Himalayan states (Uttarakhand and Himachal Pradesh) of India. The significant variations were observed in cone weight, cone length, cone width, seed length, seed width, seed weight, seed germination, radicle length, and plumule length among different seed sources of the species. Significant positive correlation between seed weight, cone weight and cone width showed that seed weight in the species depend more on the cone size. Seed germination was also positively correlated with seed weight, cone weight and radicle length in the study. The estimates of variability with regard to genetic parameters for seed weight, seed germination, cone length, cone width, cone weight showed wide range of variation in the study. Seed weight showed high heritability values coupled with maximum genetic gain. Traits with such values indicate presence of good amount of heritable additive components and are under strong genetic control. The findings of the study revealed that seed sources expressed both phenotypic and genotypic differences in the seed and cone traits which might be due to the differences in genetic make up of various seed sources and environmental factors i.e. genotypic and environmental interaction. The study suggests that the seed weight should be given the top priority for the further improvement of this species.

Keywords: seed sources; seed traits; heritability; genetic gain; improvement.

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Introduction

Pinus wallichiana, commonly known as blue pine, is indigenous to the Himalayan mountain regions, growing naturally over large areas in many countries. It has a large natural distribution with longitudinal and latitudinal range from 69° to 75° East and 26° to 36° North, respectively. It is abundant on steep slopes and its altitude ranges from 1,500 to 3,600 m. This species is extensively found in Afghanistan, Pakistan, India, Nepal, Bhutan, Myanmar and China (Troup 1921; Critchfield and Little 1966), and it is an important component of the middle and high-altitude Himalayan forests in these regions. *P. wallichiana* is a five-needle pine and gained world-wide attention for its resistance to blister rust among white pines. Its timber is extensively used for a variety of purposes including construction and railway sleepers. Blue pine forests are important for social and economic uplift of people living in mountain regions as well as for protection of steep slopes (Siddiqui 1980).

The occurrence of blue pine over a wide geographical range encompasses a great diversity of edapho-climatic conditions in its habitat, which is expected to be reflected in the genetic constitution of its population, thereby, offering an opportunity to study the variations present in the species. In addition, out crossing of mating system with wind pollination in this species also provides an opportunity of presence of high genetic variations in these forests. In a study in Bhutan, the level of genetic diversity in natural populations of this pine was found to be relatively high and the degree of genetic differentiation low compared with those of other pines (Lee et al. 1998). Dogra (1972) described seven broad provenance types in *P. wallichiana*; four-highl evel type in the inner dry & outer monsoon regions in the western Himalayas and three-low level types inner dry, middle moist & outer wet types in the eastern Himalayas,. Kriebel and Dogra (1986) carried out a seed source trial of this species in Ohio and reported significant intra-specific variation in the species and suggested further investigations using seed sources from its extensive range of distribution.

The assessment of genetic variability is a key to progress in tree improvement and is a useful tool in determining the strategies for improvement and breeding of any species. The present investigation was undertaken to ascertain the variability, heritability and genetic gain present in cone and seed traits of 17 seed sources of blue pine from wide range of occurrence in west Himalayan states.

Materials and methods

Sample collection and measurement

The geographical position and altitude of different seed sources of *P. wallichiana* used in the experimentation are given in Table 1.

Table 1. Geographical location of seed sources of blue pine.

S.N.	Seed Source	State	Longitude (°E)	Latitude (°N)	Altitude (m)
1	Jagat Sukhi	Himachal Pradesh (H.P.)	78.08	31.16	2250
2	Panthaghati	H.P.	77.36	31.10	2400
3	Kumarsain	H.P.	78.53	31.05	2532
4	Naggar	H.P.	77.69	31.05	2585
5	Nathan	H.P.	77.48	31.48	2600
6	Kufri	H.P.	78.23	30.15	2622
7	Theog	H.P.	78.55	30.05	2635
8	Kandiali	H.P.	78.87	29.65	2640
9	Kotgarh	H.P.	7831	31.50	2750
10	Nichar	H.P.	78.15	31.35	2768
11	Sarahan	H.P.	77.57	32.03	2800
12	Chamba	H.P.	76.32	32.26	2870
13	Nandprayag	Uttarakhand (UK)	79.50	30.70	914
14	Barkot	UK	78.14	30.48	1524
15	Nagnath	UK	79.23	30.15	1615
16	Joshimath	UK	79.53	30.24	1890
17	Gangotri	UK	77.34	28.53	3140

The mature cones of *P. wallichiana* were collected from 17 seed sources (seed stands) from the west Himalayan states of Himachal Pradesh and Uttarakhand during October–November 2005. Cones were collected from randomly selected 10 average trees located at least 100 m apart from each other at each of the location. Cone and seed parameters of all the seed sources were measured following International Seed Testing Association (ISTA 1993) rules at Forest Tree Seed Laboratory, Forest Research Institute, Dehradun. Measurements were recorded for cone size and fresh weight of cone using 20 randomly drawn mature cones from each location. However, the cones of four seed sources were already open at the time of collection, so cone parameters could not be measured. The seed dimensions i.e. seed length and seed width of 25 seeds were recorded in millimeters using electronic caliper. Seed weight was taken of the pure seed fraction in replications and expressed as the 1000 seed weight. Seeds of each location were germinated in Petri dishes on moist blotters at 25°C in germinator in four replications of 50 seeds each. Germination count was recorded daily and germination test was run for 28 days. Seedlings parameters i.e. root length and shoot length of 20 seedlings were measured when the germination trial was completed.

Statistical analyses

The collected data on various aspects were analyzed statistically for variability, correlation and genetic superiority using SPSS package (Anon 2007).

Analysis of variability

The observations were computed for analysis of variance (ANOVA) as per Sukhatme and Amble (1989).

Source of variation	Degree of freedom	Mean sum of square	Expectation of mean square
Replication	r-1	Ms_r	$\sigma^2e + r\sigma^2r$
Sources	s-1	Ms_s	$\sigma^2e + \sigma^2s$
Error	(r-1) (s-1)	Ms_e	σ^2e
Total	(rc-1)	-	-

where, s and r are the number of seed sources and replications, respectively.

Variance: The genotypic and phenotypic components of variance were calculated from ANOVA as described by Burton (1952).

Genotypic variance:

$$(\sigma^2g) = (\sigma^2c - \sigma^2e)/r$$

where, σ^2c = clone mean squares, σ^2e = error mean squares and r = number of replication

Phenotypic variance: $(\sigma^2p) = \sigma^2g + \sigma^2e$

Genotypic coefficient of variance: GCV= $\sqrt{\sigma^2g}$/Mean ×100

Phenotypic coefficient of variance: PCV = $\sqrt{\sigma^2p}$/Mean ×100

Heritability: Broad sense heritability was calculated as per Lush (1949).

$$\text{Heritability } (h^2) = \sigma^2g / \sigma^2p \times 100$$

Genetic advance: The genetic advance was calculated as per Johnson et al. (1955).

Genetic advance (Gs) = $K.h^2 \sqrt{\sigma^2p}$

Where K is selection differential (2.06 at 5% selection intensity (Cotterill and Dean 1990)

Genetic gain: The expected genetic gain, in percent of mean, was calculated following (Burton and Devane 1993)

$$\text{Genetic gain} = Gs \times 100 / \text{Mean}$$

Correlation

Simple correlation coefficients (Karl pearson's) between seed, cone, germination, seedling and geographical parameters were calculated using SPSS software.

Results and discussion

The magnitude of variability in seed and cone characters in different seed sources are given in Table 2. Cone traits revealed significant differences among seed sources at 5% level of significance. The highest coefficient of variation was recorded for cone fresh weight (36.92) followed by cone length (21.09). The differences recorded may be in response to different intensities of natural selection pressure upon these traits in their natural habitat. In a leguminous species the pod, seed and germination traits were considered largely under maternal influences but were strongly controlled by micro and macro habitats, besides the age and general health of the parent trees (Isik 1986). No particular trend was observed between cone characters and geographic factors like latitude and altitude in this study, however, these were found correlated negatively with latitude and positively with altitude (Table 3). Higher cone fresh weight, cone length and cone width was recorded in seed sources of Kandiali, Kufri and Gangotri which are relatively drier areas (as rainfall is limited to three month monsoon periods) with rich soil. These sources are also occurring on comparatively lower latitude but higher altitudes. Roy et al. (2004) also recorded comparatively more cone weight and cone width in *Pinus roxburghii* seed sources from drier and lower latitude areas.

Table 2. Cone and seed characters in blue pine

Seed source	Cone fresh weight (g)	Cone length (cm)	Cone width (mm)	Seed length (mm)	Seed width (mm)	Seed weight (g)	Seed germination (%)	Radicle length (cm)	Plumule length (cm)
Jagat Sukhi	79.00	22.80	27.61	7.70	4.61	4.56	50.50	4.39	5.78
Panthaghati	44.40	12.94	21.10	8.14	4.56	3.62	67.00	5.14	5.64
Kumarsain	68.00	15.94	31.76	7.37	4.88	3.89	58.50	4.52	5.81
Naggar	62.20	21.86	34.83	8.68	4.57	3.77	50.00	3.65	5.30
Nathan	79.00	16.80	29.25	7.97	4.52	4.58	59.00	3.91	4.38
Kufri	109.50	17.12	33.78	7.25	4.83	4.80	70.50	5.70	4.32
Theog	62.80	15.84	27.77	7.73	4.32	5.12	53.50	4.57	5.75
Kandiali	114.40	19.70	35.30	7.86	4.83	6.88	85.00	5.28	5.35
Kotgarh	79.40	19.40	30.09	8.98	5.22	5.53	64.50	4.42	6.06
Nichar	77.40	19.60	35.26	8.66	4.98	4.36	70.25	4.78	6.59
Sarahan	79.40	18.34	35.48	7.76	4.77	5.15	63.00	4.43	5.67
Chamba	-	-	-	7.96	5.05	6.20	74.00	4.55	5.61
Nandprayag	-	-	-	8.09	4.73	4.07	50.00	4.25	6.08
Barkot	61.00	18.00	29.46	7.45	4.49	4.33	55.00	3.97	6.35
Nagnath	-	-	-	8.00	4.56	4.45	52.50	3.84	6.26
Joshimath	-	-	-	7.79	4.86	5.16	52.50	4.11	6.67
Gangotri	90.00	20.34	31.13	7.70	4.67	5.43	72.00	4.44	6.00
CV	36.92	21.19	15.85	7.54	7.40	18.67	21.01	17.22	15.85
SE	13.53	1.64	2.53	0.31	0.21	0.21	6.64	0.38	0.43
CD (0.05)	29.22	3.54	5.46	0.67	0.42	0.42	14.00	0.63	0.88

CV = Coefficient of variation, SE= Standard Error, CD = Critical difference

Table 3. Correlation coefficients of cone, seed and geographic parameters

Characters	Cone weight	Cone length	Cone width	Seed length	Seed width	Seed weight	Seed germination	Radicle length	Plumule length	Latitude	Altitude
Cone weight	1	0.375	0.608*	-0.228	0.421	0.762**	0.656*	0.490	-0.434	-0.397	0.389
Cone length		1	0.514	0.276	0.212	0.275	-0.094	-0.347	0.156	-0.088	0.116
Cone width			1	0.062	0.434	0.371	0.292	0.002	-0.062	-0.025	0.342
Seed length				1	0.343	-0.064	-0.005	-0.256	0.246	0.350	0.118
Seed width					1	0.399	0.475	0.294	0.172	0.338	0.275
Seed weight						1	0.646**	0.311	-0.091	-0.176	0.395
Seed germination							1	0.712**	-0.284	-0.113	0.606**
Radicle length								1	-0.327	-0.146	0.363
Plumule length									1	-0.043	-0.420
Latitude										1	0.086
Altitude											1

* Correlation is significant at 0.05 level (2 – tailed); ** Correlation is significant at 0.01 level (2- tailed)

Seed characters viz., seed length, seed width, seed weight, seed germination, radicle length, plumule length varied significantly among seed sources. In the present study the maximum seed weight (6.88 g) was observed for Kandiali which is approximately double to seed weight (3.62 g) for Panthghati area. Though there is large variation in seed weight of *P. wallichiana* yet it is not correlated with any geographic factors. In case of seed dimensions i.e. seed length and seed width, however, very little variation was found. Since the seeds were collected from different locations, from trees approximately of the same age, differences observed in cone and seed parameters may be attributed to the architectures developed as a result of adaptation to diverse environmental conditions prevailing throughout their distributional ranges. Seed size may vary due to both internal (maternal, hereditary) and external (environmental) conditions operating at the time of seed development (Harper et al. 1970). This differential development might have an adaptive advantage in local edapho-climatic conditions.

Though the seed characters are significantly different between seed sources yet they do not show any trend with latitude and altitude. However, a positive correlation between seed germination and altitude (r= 0.606) and negative correlation between seed weight and latitude was found in this study (Table 3). This observation could be explained on the basis of presence of rich soil at higher altitude forests of northwest Himalayas (Dhir 1967) and the progressive increase in the length of growing season, being occurring on lower latitudes. With this increase there is also an increment in nutrient assimilation rate finally resulting in the accumulation of more stored food material. Khalil (1986) reported similar results with the seeds of *Picea glauca* and Pitcher (1984) in black cherry (*Prunus serotina*). Significant positive correlation between seed weight and cone weight (r= 0.762), cone weight and cone width (r= 0.0.608) showed that seed weight in the species depend more on cone size. Seed germination is also found positively correlated with seed weight (r= 0.646), cone weight (r= 0.656) and radicle length (r= 0.712) in this species. These observations signify that seed vigour in *P. wallichiana* is also depending on cone characteristics.

The estimates of phenotypic and genotypic variances as well as the coefficient of phenotypic and genotypic variation of different characters of the species are shown in Table 4. The relative amount of variation in different characters can be formed by comparing the coefficient of phenotypic and genotypic variation of each character. In general, both the coefficients of phenotypic and genotypic variation were of comparable magnitude for all the characters. The heritability estimates (broad sense) were found highest in the seed weight (0.87) followed by cone length (0.47) and seed germination (0.46). The expected genetic gain by selecting the 5% best as percent of mean was found to be maximum for seed weight (34.23%) followed by cone weight (28.00%) with minimum values in seed length and seed width. Seed weight provides sufficient amount of genetic variability as it is evidenced from GCV and PCV estimates in combination with heritability. Johnson et al. (1989), Singh and Uppal (1977), Volkar et al. (1990) and Singh and Chaudhary (1993) reported that the heritability estimates along with estimates of expected genetic gain is more useful than the heritability alone in predicting the resultant effect for selecting the best genotypes for a given trait. The consideration of seed weight in the delineation and understanding of the geographical variation has been advocated because of low plasticity of this character (Harper et al. 1970) as it is mainly influenced by maternal factors and is under strong genetic control (Lindgren 1984; Tyson 1989).

Table 4. Genotypic variance, phenotypic variance, coefficient of variation, heritability and genetic gain in cone and seed traits of blue pine

Characters	GV	PV	GCV	PCV	h^2	Gs	G. Gain
Cone fresh weight	259.64	717.13	11.95	12.28	0.36	19.86	28.00
Cone length	6.03	12.73	13.37	19.43	0.47	3.45	18.79
Cone width	8.15	24.14	9.22	15.86	0.34	3.44	11.10
Seed length	0.13	0.37	4.54	7.65	0.37	0.46	5.79
Seed width	0.02	0.128	2.99	7.56	0.16	0.12	3.00
Seed weight	0.74	0.85	17.85	19.13	0.87	1.65	34.23
Seed germination	74.44	162.55	14.00	20.69	0.46	12.08	19.60
Radicle length	0.25	0.61	11.19	17.47	0.41	0.66	14.77
Plumule length	0.33	0.80	10.01	16.00	0.42	0.77	13.41

GV = Genotypic variance, PV = Phenotypic variance, GCV = Genotypic coefficient of variance, PCV = Phenotypic coefficient of variance, h^2 =Heritability, Gs = Genetic advance, G. Gain = Genetic Gai

The estimates of variability with regard to genetic parameters for seed traits showed a wide range of variation in this study. Seed weight showed high heritability values coupled with maximum genetic gain. Traits with such values indicate presence of good amount of heritable additive components and are under strong genetic control. However, other traits showed low to moderate heritability and genetic gain. The character like seed weight had a very high genetic gain together with high heritability which indicates that high heritability obtained in this character is due to additive gene effects (Panse 1957; Keller and Linkens 1955; Misra and Saini 1988).

The variation among the seed sources is commonly used as an estimate of total genetic variation and used to calculate the degree of genetic control for a particular trait. The findings revealed that seed sources expressed both phenotypic and genotypic differences in the seed and cone traits which might be due to the differences in genetic make up of various seed sources and environmental factors i.e. genotypic and environmental interaction. The study suggests that the seed weight should be given the top priority for the further improvement of this species.

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