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Provenance variation in cone, seed and seedling characteristics in natural populations of *Pinus wallichiana* A.B. Jacks (Blue Pine) in India

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Abstract. Variability studies in different seed sources of *Pinus wallichiana* (Blue Pine) with respect to cone, seed and seeding traits were undertaken at Forest Research Institute, Dehra Dun, India under the framework of USDA pine project The studies revealed significant variability in terms of mean values, critical difference, and coefficient of variation, broad sense heritability, genetic advance and genetic gain. Most of the traits showed significant correlation with geographical factors viz. longitude, latitude and altitude. Genotypic variance (*Vg*) and genotypic coefficient of variance (*GCV*) were found to be higher than corresponding environmental (*Ve*) and environmental coefficient of variability (*ECV*) for most of the parameters except cone length, germination energy, germination energy index and collar diameter which indicate the dominance of environment on the expression of these traits. Moderate to high percentage of heritability coupled with high genetic gains for cone diameter and weight, seed/cone, seed length, thickness and weight, germination percente and value, cotyledon number, radicle and hypocotyls length, nursery germination percent & value and extension growth imply that these traits are under strong genetic control and there is ample scope for exploitation of heritable additive genetic component for further breeding and improvement in this species. UPGMA clustering analysis of various traits of cone, seed and seedling grouped all seed sources into 5 clusters with large inter-cluster distances. Clustering of geographically distant seed sources into one group revealed that distantly located sources are genetically close.
Keywords *Pinus wallichiana*, seed source variation, cone, seed and seedling characters, genotypic variance (*Vg*), genotypic coefficient of variability (*GCV*), environmental variance (*Ve*) and environmental coefficient of variance (*ECV*), heritability, genetic gain, UPGMA, geographical factors.

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Introduction

Pinus wallichinana A.B. Jacks (Blue Pine) is a highly variable species that occurs in mountainous region of lower Asia. It is one of the most commercially important and widely distributed pine species in India. The species spans a longitudinal range between 68° E to 100° E, latitudinal range between 25° N to 36° N and an altitudinal range between 1500 to 3800 m (Little & Critchfield 1969, Khan 1986). It occurs in pure or mixed forests throughout the temperate regions of the Himalayas, but prefers an optimum elevation of 2000-2500 m. In Northwest, it grows in abundance in Kashmir, Himachal Pradesh and Uttarakhand, while in Northeast, it occurs in Sikkim, Arunachal Pradesh and Meghalaya, in India. Besides timber utilization and oleoresins, the species is highly valued for its resistance to blister white rust caused by *Cronartium ribicola.*

Because of its wide distributional range with varying geographic, climatic and edaphic conditions and its long evolutionary history, a large variation within and among species is likely to occur, which may be reflected in the genetic constitution of its diverse populations. A description of the genetic structure of population and distribution of genetic variation among populations in this species is necessary to permit informed decisions of tree breeding and the conservation of plant genetic resources.

Genetic variation within and between populations is essential to exploit their improvement potential and is considered to be a substantial determinant of adaptive abilities of populations; also, it is best indicated for the knowledge of extent of variation available within the species (Subramanium et al. 1992). The significance of provenances/seed source variation studies in tree improvement is well recognized (Callaham 1964, Wright 1976, Suri 1984). These studies are necessary for scanning the available genetic variation, to utilize the best material for obtaining maximum productivity for further breeding work (Shiv Kumar & Banerjee 1986) and also help in analyzing and comparing superior and inferior characters which have great importance in breeding and/or tree improvement programmes besides preserving these variations intact for future research programme (Devgiri 1997).

Provenance variation, with respect to morphological, anatomical, physiological and biochemical traits, has been studied by many authors (Hattermer 1991, Thapliyal & Dhiman 1997, Kundu & Tigerstedt 1999, Mukherjee 2005). Seed source variation with respect to cone, seed and seedling characteristics is well documented for a number of tree species (Yeatman 1966, Khalil 1974, Venator 1974, Birot 1978, Salazar 1986, Isik 1986, Bethune & Longdon 1986, Dvorak et al. 1996, Singh et al. 1996, Thapliyal & Dhiman 1997, Roy et al. 2004, Mukherjee 2005). Mergen (1963) & Fowler & Dwight (1964) observed population differences in seed germination of *Pinus strobus.* Studies of 32 provenances of Blue Pine sampled from its natural distributional range in Pakistan were undertaken to assess genetic variation in several morphological and anatomical traits of needles, cones and seeds (Khan 2004). More recently, the attention has been focussed on morphological and physiological characteristics of seedling to assess the field performance potential of nursery stock (Donald 1982, Omi 1991).

The extent of variability across the geographical range of its growth indicates a fair degree of genetic dislocation among provenances. Geographic variation for seedling characteristics has been studied among others, in *Pinus strobus* (Stephen 1974) and *Juglans nigra* (Bey 1979). In the latter, it was correlated significantly with latitude, southern provenances growing faster and losing their leaves later. Geographical variation in nursery stage studies with respect to different seed sources have been made by Ngulube (1989) and Rehman et al. (1988), while variability in flushing due to geographical variation was summarized by Hannover (1962). Two geographical ecotypes were identified in Blue Pine on the basis of mean annual increment and growth in Pakistan, accounting for 56% variation among stands (Khan 1997, 2000).

Dogra (1972) reported that a strong representative barrier exists between the Blue Pine populations growing at low and high altitudes of both moist and dry zones. Genotypic differences can therefore, be expected to be present in these provenance types.

The present investigation was therefore, undertaken to assess the magnitude of variation in cone, seed and seedling characteristics and the extent of genetic control in these parameters. Correlation among various parameters and geographical variation existing in different populations of *Pinus wallichinana* sampled from wide range of distribution in India from states of Himachal Pradesh and Uttaranchal were also studied. Such investigation will help in the early evaluation of criteria for selection of some prominent traits both in laboratory and nursery and could be used as an index for the evaluation of provenances or progeny trials in *Pinus wallichinana* for further improvement.

Materials and methods

Geographic locations of the population. The locations of twenty different populations in Himachal Pradesh and Uttaranchal are cited in Table 1. Ten representative trees of the same age from the natural populations of *Pinus wallichinana* from North-West Himalayan ranges with considerable geographic isolation, were selected within each population as the methods adopted by FAO & Turnbull (1975). The selected trees were located minimum 100 m apart and thirty cones were collected per individual tree. The variation in cone, seed and seedling characteristics was investigated as under.

Cone and seed characteristics. 300 cones/population collected during the month of September-October were randomly mixed, and 20 undamaged cones from each of the seed sources were sampled and measured for cone

Table 1 Geographic origin of the investigated population (HP - Himachal Pradesh, UK - Uttarakhand)

S.No.	Locations	Name of Region	Latitude (N)	Longitude (E)	Altitude (m)
1	Bharmour	Chamba (HP)	32º 26’	76º 32’	2870
2	Chambi Kuper	Sirmaur (HP)	32º 05’	78º 20’	2665
3	Dodra Kwar	Shimla (HP)	32º 03’	77º 57’	2800
4	Gangotri	Uttarakashi (UA)	31º 02’	79º 05’	2700
5	Harsil	Uttarakashi (UA)	30º 46’	78º 44’	2533
6	Jubbal	Shimla (HP)	30º 57’	77º 40’	2450
7	Kalpa	Kinnaur (HP)	31º 35’	78º 15’	2768
8	Koshdaar	Shimla (HP)	31º 16’	77º 54’	2500
9	Kotgarh	Kinnaur (HP)	31º 50’	78º 31’	2750
10	Kotkhai	Shimla (HP)	31º 07’	77º 32’	2635
11	Nachan	Kullu (HP)	31º 32’	78º 08’	2250
12	Saliali	Kangra (HP)	32º 47’	76º 08’	2334
13	Sankri	Uttarakashi (UA)	30º 15’	78º 12’	1900
14	Sarahan	Kullu (HP)	31º 46’	77º 48’	2600
15	Sewai	Chamba (HP)	30º 20’	76º 26’	2900
16	Simla	Simla (HP)	31º 10’	77º 36’	2400
17	Thung	Mandi (HP)	32º 35’	76º 26’	2600
18	Tikkar	Sirmaur (HP)	31º 29’	77º 16’	2000
19	Tissa	Chamba (HP)	32º 49’	75º 50’	3000
20	Tutu	Simla (HP)	31º 12’	77º 51’	2100

size (length and width), cone fresh weight, number of scales and number of seeds per cone.The seeds were extracted manually from cones.

From each seed lot representing 20 sources, 200 composite seeds were randomly drawn and seed length, width and thickness was recorded, each in millimeter, using an electronic vernier calliper. Seed volume of 8000 seeds was recorded in cm^3, using 8 replications of 1000 seeds, each via water displacement method (Pandey 1991). Seed weight was determined for 8 replications of 100 seeds and finally transformed into 1000 seed weight.

Seed germination in the laboratory. Seed germination studies were carried out in laboratory conditions at 25 ± 3^{o} C using 4 replications of 50 seeds from each seed lot. The seeds were pre-treated with 0.01% $Hgcl_2$ for 5 minutes and germinated on moist germination paper. A seed was considered to be germinated when the radicle had emerged about 1 cm long. The data of seed germination was recorded and quantified as per ISTA (1976). The parameters studied were germination percent (%), germination value (*GV*) calculated as per Czabator (1962) procedure, mean germination time (*MGT*) according to Bonner (1983), germination energy and germination energy index (Grouse & Zimmer 1958).

The hypocotyle, radicle length were recorded at the end of germination test. The number of cotyledons were recorded at the time of emergence of seedling in Petri plates, while vigor index (total seedling length x germination %) was calculated according to Bhattacharya et al. (1991).

Seedling traits (nursery studies). Seeds sown in November in Jarmola Nursery - Tons Forest Division - in root trainers were set up in randomized block design (RBD) with 8 replications. Data was recorded on germination percent and germination value. Three seedlings per 8 replications from an individual seed source (i.e. 24 seedlings from each source) were selected and marked for measurement of extension growth and collar diameter. The length of the elongating shoot was measured at an interval of one month for a period of nine months. Collar diameter was measured at an interval of one month with the digital Vernier calliper upto nine months.

Statistical analysis. Complete randomized design was used for cone, seed and seedling traits in laboratory, while RBD was used for seedling traits in nursery. Data was subjected to analysis of variance (ANOVA) by SPSS version 6.1. The critical difference was calculated by LSD at 5% and 1% level of significance. Simple correlations (Pearson) was worked out to correlate the different traits of cone seed and seedling with geographic factors (altitude, longitude and latitude).

The variance component *Vp* (phenotypic variance), *Vg* (genotypic variance), *Ve* (environment variance). Phenotypic coefficient of variance (*PCV*), genotypic coefficient of variance (*GCV*), environment coefficient of variance (*ECV*), broad sense heritability, genetic advance and genetic gain were determined according to the Johnson et al. (1955), Burton (1952), Burton & Devane (1953).

To estimate genetic distance among different seed sources, hierarchical grouping was applied for 16 characters of cone, seed and seedling. Cluster analysis of different traits was performed through UPGMA.

Results and discussion

Cone, seed and germination traits in laboratory

The grand mean coefficient of variation, range and critical differences at 5% level of probability for cone, seed and germination traits in laboratory is shown in Table 2. The highest coefficient of variation (58.6%) was recorded for germination energy followed by germination % (36.3%). The analysis of variance revealed significant differences at 5% level among 20 provenances for all morphological traits, indicating the existence of genetic variability. Cone length, scale and seed per cone were recorded maximum in Tutu seed source though cone diameter and weight were observed maximum in Jubbal population. Minimum values for the same were shown by Dodrakwar except cone diameter which was lowest in Kalpa. This variation in cone traits among seed sources is probably due to influence of different intensities of natural constraints prevailing in their geographical origin. Sagawal (1984) and Sehgal (1994) reported significant variations in length, width, fresh weight and number of seeds per cone in different seed sources of *Pinus roxburghii*. Similar variations in cone traits were also reported in 63 seed sources of *Pinus roxburghii* by Mukherjee (2005). Variation in cone size could be partitioned approximately equally between location, trees within location and among cones within tree. This suggests a combined control of genotype and environment such as climate, soil, position in the crown and local density (Lester 1969). Burdon & Lou (1973) found the genetic control over different cone traits of *Pinus radiata*. Cones of higher weight are significant in obtaining higher yield and quality seeds (Maheshwari & Konar 1971). The traits are interdependent and are genetically controlled.

Seed traits, namely seed length, width, weight, thickness, volume and germination parameters vary significantly among seed sources. Maximum value for seed length (11.39 mm) and seed width (6.34 mm) was observed in Bharmour and Tissa provenance respectively, while minimum values of seed length (9.1 mm) and seed width (4.36 mm) were recorded for Dodrakwar and Kalpa respectively. The maximum seed weight (75 gm) was observed for Jubbal, which is more than twice the seed weight of Dodrakwar (34 gm). Seed weight showed much variation with a wide range and correspondingly higher coefficient of variation (14.95)(Table 2). The larger seed size and weight has been generally observed to produce faster germination and initial seedling growth. The variation in seed size may be due to both internal (maternal, hereditary) and external (environmental) conditions operating at the time of seed development (Harper et al.1970) and advantageous for wide range of adaptabil-

Table 2 Variability estimates for cone, seed and seedling traits in the laboratory

Sl. No.	Parameters	Range	Mean	CD	CV%
1.	Cone length (cm.)	15.24 - 20.45	17.06	1.37**	12.90
2.	Cone diameter (cm)	3.46 - 5.48	4.20	0.69*	10.46
3.	Cone weight (g)	64.93 -138.22	103.12	12.98**	16.50
4.	Scale per cone	102.85 -166.70	133.22	18.32*	13.10
5.	Seeds per cone	45.00 - 76.90	57.18	8.32*	14.48
6.	Seed length (mm)	9.10 - 11.39	9.86	0.44**	8.37
7.	Seed width (mm)	4.36 - 6.34	5.36	0.41**	7.81
8.	Seed thickness (mm)	2.64 - 4.34	3.40	0.23*	9.97
9.	1000 seed weight (g)	34.56 - 75.15	55.79	8.38**	14.95
10.	Seed volume (1000 seeds cc)	17.25 - 26.53	21.30	1.98*	11.85
11.	Germination %	16.00 - 80.67	59.13	22.48**	36.36
12.	Germination value (GV)	1.41 - 40.97	21.49	11.33**	30.31
13.	Germination Energy	10.14 -178.25	99.10	35.27**	58.61
14.	Germination Energy Index	5.80 - 74.80	54.68	22.49*	28.04
15.	Germination period (days)	13.30 - 19.30	16.80	4.20*	7.50

Note: ** - significant at $p < 0.01$, * - significant at $p < 0.05$

ity. Seed size has been found to regulate germination and subsequent seedling growth in many species (Baldmin 1942, Langdon 1958, Williams 1967, Kandya 1978, Devagiri 1997, Singh 1998).

Considerable variation prevailed between the sources in all germination parameters and seedling characteristics in laboratory. Highest germination percent (80.67) and germination value (40.97) were found for Bharmour while maximum germination energy (178.25) was observed for Jubbal. Mean germination period was maximum (19.3 days) for Dodrakwar while it took only 13.3 days for Jubbal seed to germinate. Variation in germination of seed sources has been reported in *Acacia mangium* (Salazar 1989), *Pinus brutia* (Isik 1986), *Betula ermanie* (Shembreg & Protemkin 1987*), Pinus greggi* (Dvorak et al.1996) *Acacia catechu* (Ramachandra 1996) and *Pinus roxburghii* (Roy et al. 2004). In general pod, seed and germination traits are supposed to be inherited characters influenced by age, growth, micro and macro habitats of the parent tree (Isik 1986). Germination value, an index combining speed and completeness of germination was influenced by seed size and weight (Baldwin 1942, Czabator 1962, Dunlop & Barnett 1984). Larger seed germinate faster and more completed than smaller one probably due to more endosperm nutrient pool (Kandya 1978). In this case, Jubbal seed source had shown better germination energy, germination value and lower germination time as compared to others. Significant variation in germination value among seed sources found in our study is in conformity with the findings of Singh and Singh (1981) in Fir and Spruce, Mathur et al. (1984) in *Acacia,* Bahugana et al. (1989) in *A. falactaria* and Mukherjee (2005) in chir pine.

Significant differences with respect to different seedling traits viz., hypocotyle and radicle length, no. of cotyledons, and vigour index in laboratory were observed for various seed sources (Table 3). Most of the maximum values of different parameters were depicted by Jubbal. Vigour index significantly different among provenances with values ranging from 89.28 to 659.23 and a coefficient of variation of 48.62% (Table 3).

Source of variation in seed and seedling traits have been well documented in a number of trees species viz. *Pinus controta* (Birot 1978) *Pinus ellioti* var. densa (Bethune & Langdon 1986), *Picea abies* (Aleksandrov 1985), *Pinus greggii* englim (Dvorak et al. 1996), *Dalbergia sissoo* (Devagiri 1997) *Santalum album* (Sinduverendra et al. 1999) and *Acacia nilotica* (Gera et al. 2000), *Cedrus deodara* (Hussain 2002). In fact, edaphic climatic factors of the place of origin are the most crucial factors affecting seed traits. Variations in seed parameters in *P. wallichiana* may be attributed to different genetic architectures developed as a result of adaptation to diverse environmental conditions exiting throughout their distributional range (Salazar & Quesda 1987) starting from northeastern part of India to the state of Jammu and Kashmir.

It was found that the seed source with more cone weight, seed weight and size showed better germination performances. Physiologically efficient cotyledons play a major role in seedling growth, development and establishment.

Table 3 Variability estimates for seedling traits in the laboratory

Sl.No.	Parameters	Range	Mean	CD	CV%
1.	Hypocotyl length (cm)	3.10 - 5.05	4.07	0.29**	10.17
2.	Radicle length (cm)	2.24 - 3.48	2.88	0.43**	10.31
3.	Total length of seedling (cm)	5.58 - 8.31	6.95	0.54**	20.80
4.	Number of cotyledons	10.08 - 13.70	11.41	0.68*	9.37
5.	Vigor index	89.28 - 659.23	422.10	58.63**	48.62

Note: ** - significant at $p < 0.01$, * - significant at $p < 0.05$

Food reserves stored in endosperm/ cotyledons of gymnosperm are utilized during seed germination and early seedling growth (Marshall & Kozlowski 1974, 1976). The seed sources having more cotyledons (12-13) viz. Tutu, Jubbal, Tisa and Gangotri showed vigorous growth in laboratory as well as in nursery conditions.

The importance of cotyledonary photosynthesis for normal seedling development has been established for some woody gymnosperms (Kozlowski & Borger 1971, Sasaki & Kozlowski 1968, 1969, 1970). Reduced photosynthesis in *P. resinosa* cotyledons inhibited expansion of primary needles (Sasaki & Kozlowski 1970). Thus correlation of cotyledonary growth with a large number of seeds and seedlings characters is obvious.

Nursery traits. Nursery data revealed statistically significant differences among seed sources at 5% level of significance with respect to nursery germination, germination value, extension growth and collar diameter (Table 4). The estimates of variability with regard to different nursery traits depicted wide range of variation. Germination percent and germination value ranged from 19.8 to 68.7% and 8 to 34.2% respectively, with a coefficient of correlation of 14.05% and 23.12%, respectively (Table 4). Nursery germination percent and value was observed maximum in Tutu and Jubbal seed source. However, minimum values of the same were observed in Dodrakwar and Chambi kuper. Jubbal seed source excelled all other source in height, followed by Tutu and Tikker sources. Highest collar diameter was seen in Tikker source, followed by Nachan and Thung seed sources. However, minimum height and collar diameter were observed in Kotkai and Kalpa sources respectively.

Seedlings of different seed sources, when grown under identical environment conditions, often display different patterns of shoot growth (Dorming 1979, Rehfeldt & Wycoff 1981, Soresen 1979). In the present investigation, when seed of all seed sources are raised under common nursery conditions, the environmental influences are nullified, variation thus obtained in nursery growth being interpreted as genetic. Sneizko and Stewart (1989) opined that provenance and within provenance variation in nursery traits are essentially genetic in nature. Seed germination and seedling growth parameters are interdependent and all are governed by the genetic make up, environmental influences and seed traits (Dunlop & Barnett 1984, Pathak et al.1984).

Variance and coefficient of variability. Partition of total variance in different cone, seed and germination traits are presented in Table 5. Genotypic variance (*GV*) and genotypic coefficient of variability (*GCV*) for most of cone and seed parameters were found to be higher than corresponding environment variance (*EV*) and environmental coefficient of variance (*ECV*).

Cone traits. Perusal of data (Table 5) depicted that the maximum phenotypic variance was for the number of seeds per cone (420) while the minimum value was recorded for cone diameter (1.31). The genetic variance in cone traits varied from 339.2 (cone weight) to 0.53 (cone length). For the environment variance, the maximum value was recorded for seeds per cone (152.04) while the minimum value was for cone diameter (0.15). With respect to coefficient of variability , the pheno-

Table 4 Variability estimates for seedling traits in nursery

Sl.No.	Parameters	Range	Mean	CD	CV%
1.	Nursery germination %	19.80 - 68.70	47.60	11.70**	14.05
2.	Nursery germination value	8.00 - 34.20	20.80	5.54**	23.12
3.	Extension growth (cm.)	11.30 - 16.20	13.80	20.20**	9.23
4.	Collar diameter (mm)	6.28 - 8.88	7.40	0.93*	8.79

Note: ** - significant at $p < 0.01$, * - significant at $p < 0.05$

Table 5 Variances and coefficient of variability for cone, seed and seedling traits in the laboratory

Parameters	*Vp*	*Vg*	*Ve*	*GCV*	*PCV*	*ECV*
Cone length (cm.)	1.50	0.53	0.97	4.27	7.17	5.78
Cone diameter (cm)	1.31	1.16	0.15	8.05	8.56	2.89
Cone weight (g)	386.81	339.20	47.61	17.86	19.07	6.69
Seeds per cone	420.00	268.40	152.04	30.42	38.12	22.92
Scale per cone	136.36	64.91	67.22	9.30	13.00	9.12
Seed length (mm)	1.38	0.95	0.43	9.88	12.00	6.60
Seed width (mm)	0.27	0.05	0.22	4.00	9.64	8.77
Seed thickness (mm)	0.18	0.16	0.03	11.50	12.47	4.65
1000 seed weight (g)	154.56	143.90	10.65	23.34	24.19	6.35
Seed volume (1000 seeds cc)	12.50	4.04	8.46	9.45	16.61	13.68
Cotyledon number	1.22	0.61	0.60	6.97	9.82	6.92
Shoot length	0.44	0.36	0.08	14.70	16.30	6.95
Root length	0.20	0.14	0.06	12.90	15.50	8.50
Germination %	309.60	205.90	103.70	24.30	29.80	17.20
Germination value (*GV*)	16.88	12.24	4.64	16.27	19.12	10.02
Germination Energy	328.39	73.24	255.15	9.39	18.28	16.11
Germination Energy Index	264.50	78.67	185.88	16.22	29.84	24.43

Note: *Vp* - phenotypic variance, *Vg* - genotypic variance, *Ve* - environment variance, *GCV* - Genotypic coefficient of variance, *PCV* - phenotypic coefficient of variance, *ECV* - environment coefficient of variance.

typic, genotypic and environmental coefficient of variability was maximum in number of seeds per cone 38.12, 30.42 and 22.92 respectively while the minimum *PCV* (7.17) and *GCV* (4.27) was recorded for cone length and minimum *ECV* (2.89) for cone diameter.

Seed traits. In case of seed traits maximum phenotypic, genotypic and environmental variance was recorded for seed weight while minimum phenotypic and environmental variance was observed for seed thickness (0.18 and 0.030) respectively and minimum genotypic variance was observed in seed width (0.05). Maximum phenotypic and genotypic coefficient of variation was observed in seed weight (24.9, 23.3) respectively while maximum environmental coefficient of variation was observed in seed volume (13.68), though minimum *PVC* & *GVC* was observed in seed width (9.6, 4.0) respectively and minimum *ECV* was observed in seed thickness (4.65) (Table 5).

Most of the characters of cone and seed morphology are controlled very strongly by genotype of individual trees. Environmental factors, which vary with location and populations within locations have only a small effect (Khalil 1974).

Germination traits. Perusal of data (Table 5) depicted that the maximum phenotypic and environmental variance was for the germination energy (328.4, 255.2) respectively, while the minimum value was recorded for root length (0.2, 0.06) respectively. The genetic variance in germination traits varied from 205.9 a to 0.14 (root length). With respect to the coefficient of variability, the phenotypic and environmental coefficient of variability was maximum in germination energy index (29.8 and 24.4) respectively, while the minimum *PCV* (9.82) and *ECV* (6.92) were recorded for cotyledon number. Maximum genotypic coefficient of variation was observed in germination value (16.72). Genotype has a strong influence on vigor of the seed (Schmidt 2000). The minimum value for the same was observed in cotyledon number (6.97). Genetic variability within and between populations has been formed by natural selection to produce populations that are physiologically attuned to a specific range of environmental conditions. As a result, long term growth development reproduction and survival depends on individual being genetically suited to the environment in which they grow. In our studies, the seed vigor i.e. germination percent and value exhibited quite high values of *GCV*, with 36.36 and 30.31% coefficient of variation. The extent of high genetic control over germination in coniferous seed has been reported high due to the high proportion of maternal genotype in their seed structure (EL-Kasaby et al. 1992).

Seedling traits (nursery). In case of seedling traits (nursery) maximum phenotypic, genotypic and environmental variance was observed in nursery germination percent (214.06, 174.54 and 39.56) respectively though minimum genotypic variance was observed in collar diameter (2.13) and minimum phenotypic and environmental variance was reported in extension growth (1.24, 4.87) respectively. With regard to coefficient of variability, maximum *PCV* and *GCV* was observed in germination value (36.4, 33.6) respectively while maximum *ECV* was observed in collar diameter (33.6). However minimum values of all the three coefficients were observable in extension growth (14.1, 16.3, 8.2) respectively (Table 6).

Estimates of genetic component for cone, seed and germination traits. Genetic parameters work out were heritability (broad sense), genetic advance (at 5% selection intensity) and genetic gain (genetic advance as percent of mean) (Table 7 and 8). In cone traits,

Table 6 Variances and coefficient of variability for seedling traits in the nursery

Parameters	*Vp*	*Vg*	*Ve*	*GCV*	*PCV*	*ECV*
Nursery germination	214.06	174.57	39.56	27.81	30.80	13.24
Germination value	57.04	48.51	8.53	33.56	36.39	14.07
Extension growth (cm)	4.87	3.63	1.14	14.11	16.34	8.24
Collar diameter (mm)	8.42	2.13	6.19	19.70	29.00	33.63

Note: *Vp* - phenotypic variance, *Vg* - genotypic variance, *Ve* - environment variance, *GCV* - Genotypic coefficient of variance, *PCV* - phenotypic coefficient of variance, *ECV* - environment coefficient of variance.

Table 7 Estimate of genetic components for cone, seed and seedling traits in the laboratory

Parameters	Heritability	Genetic advance	Genetic gain (%)
Cone length (cm.)	35.33	0.89	5.21
Cone diameter (cm)	88.50	2.08	45.90
Cone weight (g)	87.69	35.52	61.06
Seeds per cone	64.00	28.00	50.25
Scale per cone	47.14	11.31	12.60
Seed length (mm)	67.86	1.65	16.73
Seed width (mm)	18.50	0.197	3.69
Seed thickness (mm)	86.11	0.75	22.00
1000 seed weight (g)	93.10	23.01	44.78
Seed volume (1000 seeds cc)	32.32	2.35	13.40
Germination %	66.50	24.10	40.86
Germination value (*GV*)	72.53	6.92	28.47
Germination Energy	22.30	8.32	8.38
Germination Energy Index	29.74	9.96	18.21
Cotyledon number	50.32	1.15	10.23
Shoot length (cm)	81.80	1.17	27.40
Root length (cm)	70.00	0.64	22.30

Table 8 Estimate of genetic components for seedling traits in the nursery

Parameters	Heritability	Genetic advance	Genetic gain (%)
Nursery germination	81.55	24.57	51.74
Germination value	85.04	13.23	63.74
Extension growth (cm)	74.54	3.38	25.09
Collar diameter (mm)	25.20	0.93	12.50

heritability was observed maximum (88.5) in cone diameter. Maximum genetic gain and genetic advance of 35.52 and 61.06 respectively were reported for cone weight. However, the cone length revealed minimum 35.5 percent of heritability besides minimum values for genetic advance (0.89) and gain (5.21) (Table 7).

For seed, traits highest value of heritability, genetic advance and genetic gain was observed for seed weight (93.1, 23.0, and 44.8) respectively, while seed width reflected least values for all the three traits (18.5, 0.197, 3.69) respectively.

In germination traits heritability was observed maximum 81.8 in shoot length while it was observed minimum for germination energy (22.3). Maximum genetic advance and genetic gain 24.1 and 40.9 respectively was reported for germination percent while minimum values for the same was observed in root length (0.64) and germination energy (8.38) (Table 7).

In seedling traits (nursery), maximum heritability (85%) was observed in germination value and minimum in collar diameter (25.2%). Maximum genetic advance and genetic gain 24.57 and 63.74 respectively, were observed in nursery germination percent and germination value respectively , though minimum values for both were observed in collar diameter (0.95 and 12.5) respectively (Table 8).

Extension growth revealed 58% heritability but with very low genetic gain of 195. Similar results were also observed for scale per cone, seed dimensions for seed length and thickness, seed volume and germination characters like cotyledon number, hypocotyle and radicle length. Despite good values of heritability, these traits did not show expected genetic gain which could be due to more non-additive genetic effects than additive genetic effects.

Partitioning the total phenotypic variance (V_p) of each trait into heritable (*vg*) and non heritable component is helpful in determining the proportion of heritable variation that is exploited for selection of prominent traits. Estimation of heritability is useful as gross indicators of the possibility of selection for one or more traits (Namkoong et al. 1966). Moderate to high percentage of heritability with some intensity of gain as exhibited by cone diameter, cone weight, seed per cone, laboratory and nursery germination percent, germination value and seed weight suggest that these traits are highly genetic in origin with good amount of heritable additive genetic component and could be used as criterion for the selection of best population in this species for its improvement. Johnson et al. (1995) observed that high heritability value along with genetic gain in traits is more adequate and accurate for selecting best individuals from best Provenances. This is in conformity to the findings of Hooda and Raj Bhadur (1993) in *Leucaena leucocephala*, Bhardwaj et al. (1983) in *Dioscorea deltoidea* and Mukherjee (2005) in *Pinus roxburghii*. High heritability values coupled with low genetic gain were seen in scale per cone, seed thickness, cotyledon number, and hypocotyle and radicle length indicating that these traits have more of non additive genetic components than additive ones.

Correlation matrix. Significant correlation were found between morphological traits of cone with other important traits like cone length which is positively correlated with seed weight (0.723), cone diameter which is positively correlated with seed volume (0.792) and germination percent (0.625), cone weight having positive correlation with germination value (0.793), number of seed per cone (0.831) which is positively correlated with number of scale per cone (0.937). All the three morphological traits of seed viz. seed weight, seed length and seed width show significant correlation among themselves (Table 9). This type of relationship has been shown earlier by Salazar 1989, Sneizko and Stewart 1989, Bagchi and Dobriyal (1990) and Baker (1972) revealing thus that these traits are independent and genetically controlled. Shiv Kumar and Banerjee (1986) also reported significant correlation among seed weight, speed and uniformity of germination in *A. nilotica*. Significant correlation of seed length and weight with germination percentage and germination value in *P. roxburghii* was reported by Mukherjee (2005). Number of cotyledons was highly correlated with seed weight, seed length, width, germination value, extension growth and collar diameter (Table 9).

A strong positive relationship of different growth parameters with seed size, weight and seed vigor was revealed (Table 9). A positive correlation of collar diameter with seed and seedling vigor depicted that seed sources which germinated fast and adapted to the local environment tended to show greater collar diameter as compared to seed sources with less vigorous seeds. Similar relationship was obtained in *C. deodara* (Hussain 2002) and *Pinus roxburghii* (Mukherjee 2005).

Genetic divergence. Clustering of 20 seed sources was done using 18 traits of cone, seed and seedling viz. cone length, diameter, weight, and seed/scale per cone, seed length, width, weight, volume, laboratory germination %, germination energy and germination period, hypocotyle length, cotyledon number, nursery germination percent and germination value, extension growth and collar diameter. A total of 5 clusters were obtained (Table 10). Cluster 3 was the largest consisting of 7 seed sources followed by cluster 4 having 6 seed sources. Thus for any tree improvement progamme it is appropriate to select preferably seed sources from two divergent clusters, however crossing of very diverse genotypes may not yield proportionate heteriotic response. In our study though cluster 4 and 1 are highly divergent but the average performance of seed sources in Cluster 1 is not good. Seed sources of cluster 3, 4 and 5 are best genetically divergent genotypes with overall good performance. Though cluster I and *V* are divergent yet average performance of cluster *V* is higher.

Inter cluster distance. Inter cluster distance for five clusters is presented in Table11. The maximum inter cluster distance of 131.84 units was recorded between cluster 1 and 5 followed by cluster 2 and 5 while cluster 2 had least genetic distance with cluster 3. The seed sources from geographically distant zones have been grouped in one cluster indicating that pattern of genetic nearness is not dependent on geographical nearness. *Pinus wallichiana* is highly out crossed species and large volume of gene flow is expected.

Correlation between parameter and geographical origin. Simple correlation was calculated between various parameter studied and geographic factors like altitude, longitude and latitude which is presented in Table 12.

Statistically negative correlation was observed between altitude and most of the cone, germination and easy growth traits. Majority of seed characters also did not exhibit any significant correlation with altitude.

Most of the cone, germination and nursery traits were negatively correlated with latitude though germination period were positively correlated (0.497). This could be attributed to the fact that cone size and weight often increase with geographic changes that are associated with the increase in the length of growing season for example, decreasing latitude (Langlet 1938, Sziklai 1969) and decreasing elevation (Atay 1959, Hermann 1968). Khalil (1986) have reported N-S trend in most of cone parameters of *Picea glauca*. Hussain (2002) also

Table 9 Simple correlation between cone, seed and seedling traits of *P. wallichiana*

Parameters	Alt	Lati	Longl	CL (cm)	CD (cm)	CW (g)	SPC	Seeds PC	SL (mm)	SW (mm)	ST (mm)	1000 SW (g)	SV (cc)	GP	GV	GE	GEI	G. period	LH	NC	VI	PSA	ASA	NGP	NGV	Eg	CD
Alt	1																										
Lati	0.441	1																									
Longl	-0.380	-0.702	1																								
CL (cm)	-0.536*	-0.457*	-0.423*	1																							
CD (cm)	-0.381*	-0.415*	-0.036	0.705**	1																						
CW (g)	-0.595**	-0.446*	-0.461*	0.855**	0.635**	1																					
SPC	-0.467*	-0.257	-0.142	0.947**	0.746**	0.892**	1																				
Seed PC	-0.527*	-0.203	-0.062	0.959**	0.703**	0.831**	0.937*8	1																			
SL (mm)	-0.070	-0.140	-0.168	0.507*	0.722**	0.675**	0.661**	0.541*	1																		
SW (mm)	-0.077	0.124	0.022	0.450*	0.626**	0.499*	0.547*	0.550*	0.642**	1																	
ST (mm)	0.016	-0.065	-0.196	0.459*	0.635**	0.651**	0.621**	0.534*	0.866**	0.851**	1																
1000 SW (g)	-0.586**	-0.495*	-0.422*	0.723**	0.780**	0.722**	0.766**	0.686**	0.817**	0.591*	0.680*	1															
SV (1000 seeds) (cc)	-0.219	-0.200	-0.144	0.718**	0.792**	0.749**	0.777**	0.685**	0.763**	0.687**	0.745**	0.806**	1														
Ger. %	-0.449*	-0.491*	0.100	0.637**	0.652**	0.643**	0.711**	0.654**	0.712*8	0.642**	0.694**	0.784**	0.770**	1													
Ger. Value	-.0.414*	-0.495*	-0.059	0.677**	0.728**	0.793**	0.792**	0.703**	0.821**	0.662**	0.795**	0.789**	0.850**	0.906**	1												
Ger. Energy	-0.446*	-0.400*	-0.013	0.691**	0.796**	0.767**	0.794**	0.710**	0.799**	0.621**	0.740**	0.7587**	0.851**	0.884**	0.978**	1											
Ger. Energy index	-0.426*	-0.218*	0.045	0.672**	0.684**	0.686**	0.736**	0.700**	0.686**	0.679**	0.701**	0.767**	0.795**	0.972**	-0.904**	0.877**	1										
Ger. period	0.491*	0.497*	0.109	-0.742**	-0.801**	-0.753**	-0.811**	-0.756**	-0.791**	-0.644**	-0.754**	-0.770**	-0.794**	-0.779**	0.781**	-0.922**	-0.755**	1									
LH	-0.296	-0.403*	0.069	0.737**	0.848**	0.713**	0.740**	0.744**	0.803**	0.627**	0.715**	0.807**	0.839**	0.760**	0.598*	0.798**	0.802**	-0.775**	1								
NC	-0.208	-0.423*	-0.088	0.719**	0.726**	0.680**	0.726**	0.654**	0.679**	0.448*	0.540*	0.652**	0.657**	0.550*	0.929**	0.634**	0.551*	-0.660**	0.768**	1							
VI	-0.477*	-0.481*	0.123	0.688**	0.754**	0.712**	0.741**	0.704**	0.803**	0.665**	0.747**	0.831**	0.825**	0.962**	-0.274	0.915**	0.951**	-0.839**	0.889**	0.699**	1						
Per (Sp. A)	0.520**	-0.021	0.058	-0.440*	-0.425	-0.275	-0.247	-0.288	-0.039	0.288	-0.026	-0.396	-0.351	-0.281	0.018	-0.288	-0.325	0.264	-0.236	-0.217	-0.272	1					
Acp (Sp. A)	0.436*	-0.004	-0.441*	-0.225	-0.212	-0.180	-0.040	0.192	0.118	-0.090	0.083	-0.122	-0.069	-0.021	0.828**	0.005	-0.077	0.012	-0.245	-0.136	-0.090	0.599*	1				
NGP	-0.579**	-0.487*	0.039	0.727**	0.716**	0.678**	-0.765**	0.730**	0.700**	0.550*	0.647**	0.793**	0.714**	0.879**	0.808**	0.849**	0.846**	-0.821**	0.768**	0.687**	-0.877**	-0.394	-0.158	1			
NGV	-0.583**	-0.460*	0.054	0.703**	0.685**	0.683**	0.750**	0.677**	0.652**	0.415	0.558*	0.734**	0.641**	0.823**	0.717**	0.843**	0.794**	-0.809**	0.699**	0.691**	-0.822**	-0.350	-0.065	0.955**	1		
E. Growth	-0.469*	-0.401*	-0.193	0.725**	0.636**	0.774**	0.743**	0.733**	0.599*	0.444	0.553*	0.612**	0.553*	0.620**	0.424	0.729**	0.663**	-0.760**	0.686**	0.757**	0.703**	-0.354	-0.139	0.763**	0.847**	1	
Collar Diameter	-0.263	-0.198	0.013	0.336	0.560*	0.549*	0.443	0.432	0.434	0.485*	0.471*	0.370	0.420	0.414		0.645**	0.530*	-0.522*	0.442	0.340	0.504*	-0.491*	-0.335	0.515**	0.552**	0.644**	1

Note: ** - significant at $p < 0.01$, * - significant at $p < 0.05$

Table 10 Clusters obtained through dendrogram analysis of different provenances of *P. wallichiana*

Cluster Number	Provenances
Cluster 1	P3, P2
Cluster 2	P15, P14
Cluster 3	P8, P9, P10, P7, P5, P17, P4
Cluster 4	P12, P19, P11, P18, P16, P13
Cluster 5	P20, P6, P1

Table 11 Intercluster distances between five clusters

Cluster No.	1	2	3	4	5
1	0.00				
2	42.81	0.00			
3	61.15	37.53	0.00		
4	87.86	45.64	48.24	0.00	
5	131.85	104.82	91.81	39.96	0.000

Table 12 Simple correlation (Pearson) between characters studied and geographical factors

Parameters	Altitude	Latitude °N	Longitude °E
Cone traits			
Cone length	-0.536*	-0.457*	-0.432*
Cone diameter	-0.381*	-0.315*	-0.036*
Cone weight	-0.595*	-0.436*	-0.461*
Scale per cone	-0.467*	-0.257	-0.142
Seeds per cone	-0.527*	-0.203	-0.062
Seed and germination traits			
Seed length	-0.070	-0.140	-0.168
Seed width	-0.077	0.124	0.022
Seed thickness	0.016	-0.065	-0.196
1000 Seed weight	-0.586**	-0.495*	-0.422*
Seed volume	-0.219	-0.200	-0.144
Germination %	-0.449*	-0.491*	0.100
Germination value	-0.414*	-0.495*	-0.059
Germination energy	-0.446*	-0.400*	-0.013
Germination energy index	-0.426*	-0.218	0.045
Germination period	0.491*	0.437*	0.109
Length of hypocotyle	-0.296	-0.403*	0.069
Number of cotyledons	-0.208	-0.423*	-0.088
Vigor index	-0.477*	-0.481*	0.132
Nursery traits (Early growth performance)			
Nursery germination percent	-0.579**	-0.487*	0.039
Nursery germination value	-0.583**	-0.460*	0.054
Extension growth	-0.469*	-0.401*	-0.193
Collar diameter	-0.263	-0.198	0.013
Enzyme activity			
Peroxidase specific activity	0.520**	0.021	0.058
Acid phosphatase specific activity	0.436*	-0.004	-0.441*

Note: ** - significant at $p < 0.01$, * - significant at $p < 0.05$

reported the same in cone length and weight in *Cedrus deodara*. Longitude was negatively correlated with cone length (-0.423) and cone weight (- 0.461). Other cone parameter did not show any significant correlation with longitude. Negative correlation of cone length and weight with longitude suggests that cone weight and cone length decreased towards the wetter high rainfall parts represented by Gangotri, Nachan and Harsil etc. High rainfall causes nutrient leaching which is reflected in stunted growth of trees and smaller size of fruits/cones (Tripathi & Banik 2001). Similar response in cone traits with respect to *Pinus rixburghii* was obtained by Roy et al. (2004) and *Pinus strobus* (Dermeritt & Hocker 1975).

Seed traits did not follow any specific trend with altitude, longitude or latitude except seed weight which interestingly showed negative correlation with all the three factors. Altitudinal variation for seed weight has been reported by Isik (1986) in *P. brutia* provenances. Birot (1978) reported the same relationship in 1000 seed weight of *Pseudostsuga menzissi* provenances. Majority of seed traits, except seed weight did not show any significant correlation with latitude.

Seed weight showed positive correlation (0.422) with longitude while other seed parameters revealed non-significant correlation. These was negative correlation of germination % (-0.48) and germination value (-0.46) with latitude. Highest germination % and germination value was revealed by those seed sources which fell in the range of 31°N to 32°N. Similar negative trend was also observed in western hemlock by Kuser & Ching (1981) and *P. glauca* (Khail 1986).

Extension growth exhibited North South trend i.e. negative correlation with latitude (-0.401). This was perhaps due to the seed sources from the lower latitude being adapted to larger continued growth starting in early spring and continuing up to later summer (Eldridge et al. 1972) leading to greater height than that of seed sources from the northern latitudes. Seed sources which germinated first adapted to the local environment, tended to show greater collar diameter as compared to those with less vigorous seeds. It was in conformity with studies of Hussain (2002) in *C. deodara* and Mukherjee (2005) in *Pinus roxburghii*.

Conclusion

Pinus wallichiana A.B. Jacks (Blue Pine) is one of the five indigenous species of India growing over wide range of geographic, climatic and edaphic conditions throughout Himalayan region.

Due to its occurrence over varying climatic conditions and a long evolutionary history, large inter and intra-specific variation is expected to be reflected in genetic constitution of its diverse populations. The present study on seed source variation of *Pinus wallichiana*, was undertaken at Forest Research Institute, Dehra Dun within the framework of USDA Pine Project. The aim of this investigation was to understand the extent and pattern of variation prevailing in 20 diverse populations of *Pinus wallichiana* with respect to cone, seed traits, germination and early growth performance and correlate it with geographical factors. Such an investigation may help in selection of superior provenance/seed source for a given site to evolve strategies for conservation, breeding and improvement in this species.

Different seed sources revealed a wide range of variability with respect to cone, seed and seeding traits in terms of mean values, critical difference, and coefficient of variation, broad sense heritability, genetic advances and genetic gains. Most of the traits showed significant correlation with geographical factors viz. longitude, latitude and altitude. The studies revealed that selection of some important characters can be made for improvement and breeding strategies.

UPGMA Clustering of geographically distant seed sources into one group revealed that distantly located sources are genetically close.

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