Journal of Genetics, Genomics & Plant Breeding 9(3) 84-100 (July, 2025)
ISSN (Online): 2581-3293 DOI: 10.63551/jggpb.vol.9.issue03.003

RESEARCH ARTICLE

Variation in the cone, seed and seedling traits of *Pinus wallichiana* A.B. (Blue Pine) in the Indian Himalaya

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Manuscript received: June2, 2025; Decision on manuscript, June 30, 2025; Manuscript accepted: July 10, 2025

Abstract

Pinus wallichiana A.B. (Blue Pine) is one of the India's five native species of Pinus, grows in a variety of climatic, topographical and edaphic conditions in the Western Himalaya. To understand the extent and variation pattern, *P. wallichiana* populations are evaluated for cone, seed and seedling characters. The results showed that the significant variation in different parameters. The study revealed that all studied traits were inherited additively, except for scale/cone and seed moisture percentage. Additionally, the significant correlation observed between germination traits of seedlings with the altitude, highlights the profound influence of provenance. Therefore, the research findings contribute to designing plantation, improvement and conservation strategies for a species, it signifies a crucial step toward sustainable management practices.

Keywords: Blue pine, seed source, cone, seedling, genetic variation

Introduction

Pinus wallichiana A.B. Jacks is commonly known as Blue Pine, belongs to family Pinaceae, and is a economically and ecologically important tree species, having extensive distribution in the Himalaya's temperate region of India (Rawat and Bakshi, 2011). This species is observed at longitudinal extent between 680E to 1000E, latitudinal extent between 250N to 360N and altitudinal extent between 1500m to 3600m; whereas, in areas receiving annual rainfall from 1000mm to 1875mm (Bhat *et al.*, 2015; Aslam *et al.*, 2017). It occurs in pure patches as well as in mixed forests and is mainly connected to conifers like *Cedrus deodara*, *Picea smithiana*, Abies alba and also in broad-leaved species like *Quercus incana*, *Quercus dilatata*, etc. Blue Pine timber is commercially important, preferred for making interior designs, door and window frames, fuel wood, light furniture, house fitments, lamina-boards, plane tables, packing cases, flush doors and railway sleepers, etc. Its needle contains turpentine that consists of 20% of oleoresin (Luna, 1996). Forests are generally exploited to fulfill the wood requirements of the regional people. In order to protect the environment, it is important to devise strategies for large-scale plantations of fast-growing indigenous and exotic tree species (Ekhuemelo *et al.*, 2016).

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Before undertaking plantation programs, the knowledge of genetic diversity, identification. of seed sources, evaluation and collection of germplasm and their maintenance is necessary for successful tree improvement programmes (Dobhal and Thakur, 2017). The genetic diversity helps in the selection of superior germplasm for increased productivity, quality and insect-pest resistance of the plant species (Kumar *et al.,* 2016; Dobhal *et al.*, 2019a). The study of seed source is a long-term process, which require extend period of time after testing one or two generations, which can be identified best seed source (Callaham, 1964) and it has a significant impact in the improvement of forest stand. To identify best species stands that can provide better adaptability, easy establishment, and higher productivity in the afforestation and reforestation programmes. The majority of the varieties at different seed sources can seen in fields and nurseries, which is genetic in nature and governed by the prevailing climatic and edaphic factors in the species distribution range. These variables control the genetic variance among individuals that come from the same and between seed sources (Swain *et al.*, 1996). Moreover, species with large geographical extent shows more variability and species with parochial geographical extent show less variability (Kraus *et al.*, 1984). Thus, the goal of the current study was done to assess the impact of various seed sources as well as the degree of genetic control in the *Pinus wallichiana* populations in the Uttarakhand state of India. The study will be beneficial in establishing the assessment criteria for the selection of noteworthy characteristics in both nursery and laboratory that might be used as a standard for evaluating provenances or progeny trials *in P. wallichiana*.

Materials and methods

Source of cone collection

The seeds were collected from ten seed sources (Table 1; Fig.1) of different districts of Uttarakhand, by selecting phenotypic superior trees, which were approx. 100m apart from each other, to exploit maximum variation in the stand (Turnbull, 1975). The hundred cones/seed source were collected in the month of September to October, 2022 and kept in cotton bags for proper air circulation and to avoid fungal infection. . The length and diameter of cone was measured using a vernier caliper, cone fresh weight was weighted in electronic balance and the number of scale/cone & seed/cone was counted manually. The cones were sun-dried for 7 to 9 days after that the seeds were manually removed from cones. The seed length with wing and without wing was count with the help of measuring scale; whereas, seed width and thickness were measure by vernier caliper. Seed weight with wing and without wing was measure by using electronic balance. The 100 seeds in five replications from each seed source was measured and recorded. Fresh weight of seed and oven dry weight of seed was measured by using electronic balance and then calculated the seed moisture content as per ISTA, 1999. The extracted seed were put in cotton bags inside the air tight jar in refrigerator for further experiment.

Seed germination

Studies on the seeds germination % were conducted in laboratory, using petri dishes and Whatman's filter paper No.1 at 200C as per ISTA, 1999. Mean daily germination, peak and germination value, germination speed (Czabator, 1962); mean germination time (Ellis and Roberts, 1981) and germination energy index (Grouse and Zimmer, 1958) were calculated. When a radicle appeared of one centimeter in length, the seed was said to have germinated.

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Table 1: Geographic origin of the investigated population of Uttarakhand

Location	Elevation (masl)	Latitude (N)	Longitude (E)
1.Garurganga	1451	$30^0$27’658"	$79^0$26’769"
2. Kasketh	1936	$30^0$06’342"	$78^0$45’247"
3. Pratapnagar	1953	$30^0$44’97"	$78^0$47’80"
4. Nagthat	2048	$30^0$36’365"	$77^0$55’742"
5.Tapovan	2068	$30^0$29’974"	$79^0$36’697"
6.Jakholi	2334	$29^0$99’285"	$79^0$09’958"
7. Ghimtali	2382	$30^0$35’320"	$79^0$09’408"
8.Harshil	2440	$31^0$01’910"	$78^0$45’385"
9.Auli	2644	$30^0$32’323"	$79^0$33’927"
10.Gangotri	3003	$30^0$59’958"	$78^0$55’202"

Fig. 1: Morphological variation of cone collected from different seed sources

When the germination phase is over, the plumule and radicle lengths were measured of 25 seedlings. By adding radicle and plumule length, the total length of seedling was obtained. The plumule: radicle was obtained by dividing the plumule length to radicle length. For morphological and germination study, randomized block design (RBD) and completely randomized design (CRD) was used, respectively.

Genotypic, phenotypic and environmental variances were calculated using the following equations of Burton and Devane,(1953).

$$\text{Genotypic Variance (Vg)} = \frac{Mt - Me}{r}$$

Phenotypic Variance (Vp) = Vg + Ve

Environmental Variance (Ve) = Me

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Where, Mt = Mean sum of square due to treatment or family; Me = Mean sum of square due to error; r = Number of replications

The genotypic, phenotypic and environmental coefficient of variation was calculated as suggested by Burton and Devane, 1953; Pillai and Sinha, 1968.

$$\text{GCV (\%)} = \frac{\sqrt{Vg}}{\bar{X}} X 100$$

$$\text{PCV (\%)} = \frac{\sqrt{Vp}}{X} X 100$$

$$\text{ECV (\%)} = \frac{\sqrt{Ve}}{\bar{X}} X 100$$

where, GCV= Genotypic coefficient of variation; PCV= Phenotypic coefficient of variation; ECV= Environmental coefficient of variation and X = Population mean of character

Heritability in broad sense was calculated as suggested by Burton and Devane, (1953); Johnson *et al*.,(1955).

$$h^2_{b.s} = \frac{Vg}{Vp} \text{ x } 100$$

The expected genetic advance at 5% selection intensity was calculated using formula suggested by Lush, 1940 and further modified by Burton and Devane, (1953); Johnson *et al.*, (1955).

$$\text{GA} = \left[\frac{Vg}{Vp}\right] \times \left(\sqrt{Vp}\right) \times K$$

where, K = 2.06 [selection differential at 5% selection intensity (Allard, 1960).

Genetic gain was worked out using methodology suggested by Johnson *et al.*, 1955 as per following formulae:

$$\text{Genetic Gain (\%)} = \frac{GA}{X} \text{ x } 100$$

Correlation coefficient

The data obtained during the course of this investigation was analyzed by applying analysis of variance (ANOVA) using the WASP 1.0. To compare the mean and standard deviation, critical difference (1 % and 5% level of significance) was calculated.

Results and discussion

Morphological variation in cone

The present results showed that the maximum value of length, diameter and weight of cone and scale and seed per cone was recorded in Harshil i.e., 22.34cm, 3.05cm, 156.32gm, 77.08 and 98.64, respectively and the minimum value found in Garurganga i.e., 13.19cm, 2.34cm, 56.43gm, 63.12 and 42.16, respectively. Among all the cone traits, the highest coefficient of variation was recorded in cone weight (11.49) and lowest in cone diameter (5.45) (Table 2). Due to changes in geographical, climatic and edaphic environments as well as its lengthy evolutionary history, genetic structures of *Pinus wallichiana* populations varied within provenance types.

Table 2. Variation on cone characteristics (Mean± SD) influenced by different seed sources

Seed source	Cone length (cm)	Cone diameter (cm)	Cone weight (gm)	Scale /cone	Seed /cone
1.Garurganga	13.19±0.40[f]	2.34±0.03[c]	56.43±3.38[e]	63.12±8.46[c]	42.16±8.59[h]
2. Kasketh	17.60±0.89[cd]	2.40±0.10[c]	71.72±9.33[d]	69.12±2.70[bc]	56.80±7.44[fg]
3. Pratapnagar	15.74±0.84[e]	2.90±0.15[ab]	78.81±10.46[d]	75.60±2.88[a]	87.48±10.55[bc]
4. Nagthat	18.59±0.83[bc]	2.76±0.12[b]	115.96±11.73[c]	71.36±3.94[ab]	54.56±7.24[g]
5.Tapovan	16.80±0.52[de]	2.90±0.10[ab]	74.16±8.63[d]	64.40±3.44[c]	85.16±7.51[cd]
6.Jakholi	20.16±0.42[b]	2.80±0.06[b]	132.64±14.01[b]	72.68±4.10[ab]	97.05±3.81[ab]
7. Ghimtali	17.48±0.99[cd]	2.87±0.08[ab]	119.60±9.88[bc]	75.56±1.19[a]	75.88±8.37[de]
8.Harshil	22.34±2.91[a]	3.05±0.41[a]	156.32±14.73[a]	77.08±6.15[a]	98.64±10.08[a]
9.Auli	17.08±1.15[cde]	2.77±0.13[b]	104.76±13.22[c]	72.20±5.90[ab]	79.92±7.53[cd]
10.Gangotri	17.02±0.83[cde]	2.74±0.10[b]	106.29±15.25[c]	73.08±3.03[a]	66.20±7.08[ef]
Mean	17.60	2.75	101.67	71.82	74.38
CV	6.98	5.45	11.49	6.57	11.00

Mean followed by the same letter is not significant at $p<0.05$

The seed source variations are carried out for tree analyzing inferior and superior germplasm for a particular trait, so as to determine the productivity of forest stands. The genetic analysis of traits of the forest has great importance in tree breeding and improvement programs as the available variation in trees could be preserved for future breeding programme (Dobhal *et al.,* 2018; Vanisri, 2024). The fact that *Pinus wallichiana* grows in a variety of climatic and edaphic environments may account for the variation in cone features found in the different seed sources, which is similar to the findings reported by Rawat and Bakshi, (2011). Further, the variations in length, diameter and weight of cone in *Pinus wallichiana* might depend upon the influence of diverse natural restrictions that are more prevalent and limiting in their geographic location, as well as more favorable environmental conditions. The distribution of the variations in cone size might be almost equal between the sites within a tree and among the trees themselves. Similar, Mukherjee, 2005 reported variations in cone traits of 63 seed sources of *Pinus roxburghii*. Moreover, the variations in cone length/diameter/weight, scale/cone and seed/cone were also stated by Roy *et al.*, 2004 in *Pinus roxburghii*, Aslam *et al.,* (2010); Singh and Thapliyal, (2012) in *Pinus wallichiana* and Singh *et al.,* (2015) in *Pinus kesiya*. Our results are consistent with the outcome by Bhat *et al.,* (2015) whom showed that cone length is positively correlated with the number of seeds.

Morphological variation of seeds

The maximum seed length with wing was recorded in Harshil (3.54cm) and the minimum value was recorded in Garurganga (2.72cm) seed source. The maximum value of seed length without wing and seed width & thickness was recorded in Gangotri seed source i.e., 8.65cm, 5.53mm and 2.78mm, respectively; whereas, minimum value was recorded in Garurganga i.e., 6.36cm, 4.46mm and 2.22mm, respectively. Maximum seed weight with wing and without wing and seed moisture % was noticed in Auli seed source i.e., 8.47gm, 8.14gm and 15.80%, respectively; whereas, minimum value was recorded in Kasketh i.e., 4.82gm, 4.43gm and 9.15%, respectively. Among all the seed characteristics, a maximum coefficient of variation was observed in seed width (7.56) and the lowest in 100 seed weight without wings (2.02) (Table 3).

Journal of Genetics, Genomics & Plant Breeding **9(3) 84-100 (July, 2025)**
ISSN (Online): 2581-3293 DOI: 10.63551/jggpb.vol.9.issue03.003

Table 3: Variation on seed characteristics (mean± SD) influenced by different seed sources

Seed Source	Seed length with wing (cm)	Seed length without wing (cm)	Seed width (mm)	Seed thickness(mm)	100 Seed weight with wing (gm)	100 Seed weight without wing (gm)	Seed moisture (%)
1.Garurganga	2.72 ± 0.21^{f}	6.36 ± 0.95^{e}	4.46 ± 0.45^{e}	2.22 ± 0.04^{c}	5.12 ± 0.12^{f}	4.85 ± 0.18^{g}	13.80±4.87
2. Kasketh	2.89 ± 0.23^{ef}	6.76 ± 0.55^{de}	5.33 ± 0.20^{abc}	2.27 ± 0.03^{c}	4.82 ± 0.06^{g}	4.43 ± 0.04^{h}	9.15±1.87
3. Pratapnagar	2.88 ± 0.17^{ef}	7.37 ± 0.67^{bcd}	5.34 ± 0.12^{ab}	2.47 ± 0.04^{b}	6.07 ± 0.16^{e}	5.63 ± 0.21^{e}	10.64±0.13
4. Nagthat	3.02 ± 0.08^{de}	7.31 ± 0.47^{bcd}	5.01 ± 0.42^{bcd}	2.47 ± 0.07^{b}	6.32 ± 0.08^{d}	5.99 ± 0.10^{d}	12.41±5.25
5.Tapovan	2.93 ± 0.26^{e}	6.89 ± 0.62^{cde}	4.95 ± 0.42^{d}	2.56 ± 0.14^{b}	6.08 ± 0.12^{e}	5.77 ± 0.18^{e}	9.80±0.29
6.Jakholi	3.33 ± 0.12^{b}	7.94 ± 0.53^{ab}	5.47 ± 0.16^{a}	2.58 ± 0.14^{b}	5.96 ± 0.07^{e}	5.58 ± 0.16^{f}	10.38±0.24
7. Ghimtali	3.19 ± 0.08^{bcd}	7.56 ± 0.31^{bc}	5.39 ± 0.07^{a}	2.27 ± 0.11^{c}	6.25 ± 0.08^{d}	6.02 ± 0.06^{d}	10.29±0.53
8.Harshil	3.54 ± 0.03^{a}	7.80 ± 0.43^{b}	4.99 ± 0.12^{cd}	2.45 ± 0.16^{b}	6.85 ± 0.12^{c}	6.42 ± 0.19^{c}	11.67±0.77
9.Auli	3.13 ± 0.06^{cd}	7.60 ± 0.43^{bc}	4.93 ± 0.19^{d}	2.53 ± 0.15^{b}	8.47 ± 0.20^{a}	8.14 ± 0.11^{a}	15.80±6.43
10.Gangotri	3.32 ± 0.12^{bc}	8.65 ± 0.48^{a}	5.53 ± 0.21^{a}	2.78 ± 0.08^{a}	7.75 ± 0.15^{b}	7.40 ± 0.08^{b}	12.92±4.99
Mean	6.21	3.09	7.42	5.14	2.46	6.37	6.02
CV	2.21	4.85	7.56	5.17	4.61	2.02	2.42

Mean followed by the same letter is not significant at $p<0.05$

Our results showed the variation in seed size which may be due to the different levels of resource availability during the seed development. This indicates that higher seed weight was noted with higher seed moisture content, which also resulted in higher seed output and quality (Maheshwari and Konar, 1971). Larger seed weight and size have often been found to quicker germination and early seedling growth. In numerous species, seed size has been found to control germination and the growth of seedlings (Kandya, 1978). Roy *et al.*, 2004 observed that variation in the seed sizes varies among all the seed sources. This was because of difference in the genetic architecture which is strongly related to the prevailing environmental condition throughout their natural habitat.

Seed germination and growth characters

This study showed the substantial influences on the germination parameters of different seed origins. The present results revealed that maximum germination %, daily germination, germination time and germination energy index was found in Gangotri seed source i.e., 80%, 0.57, 7.57 and 33.33, respectively; whereas, minimum value was found in Garurganga i.e., 35%, 0.26, 3.73 and 14.59, respectively. The maximum germination value, peak value and germination speed was recorded in the Jakholi i.e., 0.14, 0.28 and 1.55, respectively; whereas, minimum value was found in Garurganga i.e., 0.02, 0.10 and 0.49, respectively. The highest coefficient of variation was recorded in germination value (28.26) and the lowest in germination % (11.81) (Table 4). The maximum value of growth traits i.e., length of plumule, radicale and seedling and plumule-radicle ratio was recorded in the Gangotri seed source i.e., 4.95cm, 3.02cm,7.97cm and 1.80, respectively; whereas, minimum value was recorded in Garurganga i.e.,1.04cm, 1.18cm, 2.22cm and 6.77, respectively. Among all the growth parameters, the highest coefficient of variation was recorded in plumule-radicle ratio (7.26) and the lowest in seedling length (4.76) (Table 5).

Similarly, Mughal (2002) reported the substantial relationship between the seed germination, seed weight and size. Moreover, the variation observed in the different seed sources germination was in accordance with the findings of Rawat and Bakshi, (2011); Aslam *et al.*, 2017 in *Pinus wallichiana* and Xu *et al.*, (2016) in *Pinus densata*. *Manning et al.*, (2009) also showed that larger seed size showed greater survival compared to small size seeds. It has been reported that the growth, development and establishment of seedlings are significantly influenced by the physiologically efficient cotyledons (Marshall and Kozlowski, 1974). The variation in seed germination might be due to the difference in the seed size as large seed size holds more reserve food which is expected to give higher germination percentage (Kandya, 1978). The similar type of variation was also observed by Singh and Bhatt, (2006); Singh *et al.*, (2015) in Pinus kesiya, Singh and Thapliyal, (2012) in *Pinus wallichiana*, Ghildiyal *et al.*, (2009) in *Pinus roxburghii*, and Mughal and Thapliyal, (2012) in *Cedrus deodara*. As the food reserve stored in endosperm/cotyledons of gymnosperm are utilized during seed germination and early seedling growth. The cotyledonary photosynthetic activity important for the healthy seedling growth (Sasaki and Kozlowski, 1969). Further, the reduced photosynthesis of cotyledons inhibited expansion of primary needles in *P. resinosa* by Sasaki and Kozlowski (1970). Therefore, it is evident that cotyledonary growth correlates with several traits of seed and seedling.

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Table 4: Variation on germination characteristics (mean± SD) influenced by different seed sources

Seed Source	Seed length with wing (cm)	Seed length without wing (cm)	Seed width (mm)	Seed thickness(mm)	100 Seed weight with wing (gm)	100 Seed weight without wing (gm)	Seed moisture (%)
1.Garurganga	35 ± 7.07^{c}	0.02 ± 0.01^{c}	0.10 ± 0.01^{d}	0.26 ± 0.05^{c}	3.73 ± 0.78^{e}	0.49 ± 0.10^{g}	14.59 ± 1.39^{c}
2. Kasketh	37 ± 7.58^{c}	0.03 ± 0.01^{bc}	0.12 ± 0.04^{cd}	0.38 ± 0.06^{c}	3.81 ± 0.81^{e}	0.55 ± 0.13^{fg}	15.42 ± 1.47^{c}
3. Pratapnagar	62 ± 8.37^{b}	0.05 ± 0.02^{b}	0.12 ± 0.03^{cd}	0.44 ± 0.06^{b}	6.63 ± 1.07^{ab}	0.88 ± 0.11^{d}	25.81 ± 2.68^{b}
4. Nagthat	56 ± 6.52^{b}	0.05 ± 0.02^{b}	0.14 ± 0.03^{c}	0.40 ± 0.04^{b}	5.40 ± 0.72^{cd}	0.88 ± 0.13^{d}	23.33 ± 2.78^{b}
5.Tapovan	55 ± 10.0^{b}	0.04 ± 0.01^{bc}	0.11 ± 0.01^{d}	0.39 ± 0.07^{b}	6.56 ± 1.51^{ab}	0.69 ± 0.10^{e}	22.92 ± 2.38^{b}
6.Jakholi	72 ± 5.70^{a}	0.14 ± 0.02^{a}	0.28 ± 0.02^{a}	0.51 ± 0.04^{a}	5.33 ± 0.89^{d}	1.55 ± 0.08^{a}	30.00 ± 4.12^{a}
7. Ghimtali	55 ± 6.12^{b}	0.05 ± 0.01^{b}	0.14 ± 0.03^{c}	0.39 ± 0.04^{b}	5.60 ± 0.66^{bcd}	0.83 ± 0.11^{de}	22.92 ± 2.61^{b}
8.Harshil	76 ± 6.52^{a}	0.12 ± 0.02^{a}	0.23 ± 0.03^{b}	0.54 ± 0.05^{a}	7.34 ± 0.59^{a}	1.18 ± 0.11^{c}	31.67 ± 4.00^{a}
9.Auli	75 ± 6.12^{a}	0.12 ± 0.03^{a}	0.24 ± 0.05^{b}	0.54 ± 0.04^{a}	6.49 ± 0.60^{abc}	1.36 ± 0.24^{b}	31.25 ± 4.17^{a}
10.Gangotri	80 ± 6.12^{a}	0.12 ± 0.04^{a}	0.23 ± 0.04^{b}	0.57 ± 0.04^{a}	7.57 ± 0.57^{a}	1.31 ± 0.17^{bc}	33.33 ± 3.68^{a}
Mean	60.3	0.08	0.17	0.43	5.85	0.97	25.12
CV	11.81	28.26	19.02	11.89	15.05	13.85	11.82

Mean followed by the same letter is not significant at $p<0.05$

Variability and genetic studies for cone and seed traits

The maximum value of Vp and Vg was recorded in cone weight i.e., 353.51 and 420.44, respectively; whereas, minimum value of Vp and Vg was recorded in seed thickness i.e., 0.02 and 0.03, respectively. With respect to the maximum value of PCV, GCV and ECV was recorded in cone weight i.e., 32.99, 35.30 and 11.48, respectively; whereas, minimum value of PCV, GCV and ECV was recorded in seed width i.e., 6.03, 7.92 and 2.22, respectively. Results further showed that the maximum of heritability, genetic advance and genetic gain was recorded in cone weight i.e., 87.35, 58.12 and 59.10, respectively; whereas, minimum value of heritability, genetic advance and genetic gain was recorded in seed moisture % i.e., 11.59, 0.30 and7.78, respectively (Table 6). The estimation of variance and variability provides information about the degree of variation in forest trees. It determines the role of genetic and environmental factors in controlling the variation in trees (Singh and Bhatt, 2008b). The amount of heritable variation that can be used to select prominent features can be found by partitioning the total phenotypic variance of each trait into heritable and non-heritable components. Heritability estimation is helpful as broad indicators of the potential for selection for one or more features (Namkoong *et al.,* 1966). When selecting the best individuals from the best provenances, a high heritability value combined with genetic gain in attributes is more appropriate and correct (Johnson *et al.*, 1995).

Traits showed substantial genetic gain combined with high heritability, indicates the presence of additive genes action and such traits are considered best trait for selection of superior genotypes (Meena *et al.,* 2015). In the current research of cone and seed parameters, high heritability along with high genetic gain was observed for cone weight and seed/cone, which indicate superiority of these traits and should be considered for the future selection programmes. Similar findings were also observed by Chauhan and Kanwar, (2001); Roy *et al.*, (2004) in *Pinus roxburghii*, Singh and Bhatt, 2006 in *Pinus kesiya*, Aslam *et al.,* (2010); Rahman *et al.,* (2017) in *Pinus wallichiana*. Results also explained that germination and growth parameters i.e., germination % and length of plumule andseedling showed higher heritability along with higher genetic gain. The coniferous seeds include a substantial percentage of the maternal genotypes in their seed structure, there has been claimed to be high genetic control over germination (EL-Kasaby *et al.*, 1992). The genetic variation observed in various germination traits in our research are in accordance with the results of Singh and Bhatt, (2008a) in *Dalbergia sissoo*, Rawat and Bakshi, (2011) in *Pinus wallichiana* and Mohammed *et al.*, (2015) in Aquilaria malaccensis.

Variability and genetic studies in germination and growth traits

The maximum value of Vp and Vg was recorded in germination (%) i.e., 239.64 and 290.39, respectively; whereas, minimum value of Vp and Vg was recorded in daily germination i.e., 0.01 and 0.02, respectively. With respect to the maximum value of PCV, GCV and ECV were recorded in germination value i.e., 55.90, 57.10 and 30.90, respectively; whereas, minimum value of PCV, GCV and ECV was recorded in germination time i.e., 21.69, 25.93 and 4.94, respectively. Results further showed that the maximum of heritability, genetic advance and genetic gain was recorded in germination % i.e., 98.81, 28.97 and 115.16, respectively; whereas, minimum value of heritability, genetic advance and genetic gain was recorded in germination time i.e., 67.65, 0.09 and 36.75, respectively (Table 6).

Table 5: Variation on growth parameters (Mean± SD) influenced by different seed sources

Seed Source	Plumule length (cm)	Radicle length (cm)	Seedling length (cm)	Plumule-radicle ratio
1.Garurganga	1.04±0.16[g]	1.18±0.03[g]	2.22±0.16[h]	0.77±0.06[g]
2. Kasketh	1.05±0.09[g]	1.36±0.04[f]	2.41±0.11[h]	0.89±0.14[g]
3. Pratapnagar	2.22±0.12[e]	1.54±0.13[e]	3.76±0.23[f]	1.47±0.09[de]
4. Nagthat	1.81±0.07[f]	1.59±0.12[de]	3.40±0.16[g]	1.15±0.09[f]
5.Tapovan	2.18±0.09[e]	1.59±0.12[de]	3.77±0.20[f]	1.40±0.09[e]
6.Jakholi	3.30±0.19[d]	1.89±0.09[c]	5.19±0.26[d]	1.76±0.07[ab]
7. Ghimtali	2.37±0.20[e]	1.70±0.08[d]	4.07±0.24[e]	1.40±0.11[e]
8.Harshil	4.08±0.17[b]	2.66±0.15[b]	6.74±0.12[b]	1.54±0.14[cd]
9.Auli	3.62±0.24[c]	2.02±0.10[c]	5.63±0.31[c]	1.65±0.05[bc]
10.Gangotri	4.95±0.14[a]	3.02±0.13[a]	7.97±0.26[a]	1.80±0.11[a]
Mean	2.66	1.86	4.52	1.38
CV	5.87	5.71	4.76	7.26

Mean followed by same letter are not significant at $p<0.05$

Table 6: Genotypic, phenotypic and environmental variances (GCV, PCV, ECV) and genetic advance (GA), broad sense heritability (h2b.s), genetic gain (GG) for morphological traits of cone, seed and their germination and growth traits

Parameters	Vp	Vg	PCV	GCV	ECV	$h^2_{b.s}$	GA	GC (%)
Cone length	5.7	7.2	13.5	15.2	6.9	79.1	4.3	24.8
Cone diameter	0.0	0.0	7.7	9.2	5.1	69.3	0.3	13.2
Cone weight	353.5	420.4	32.9	35.3	11.4	87.3	58.1	59.1
Scale/cone	20.2	42.5	6.5	9.0	6.2	47.6	6.4	8.9
Seed/cone	345.5	412.4	24.9	27.3	10.9	83.7	35.0	47.1
Seed length with wing	0.0	0.0	7.9	9.1	4.5	75.0	0.4	14.1
Seed length without wing	0.3	0.6	8.1	11.1	7.6	53.0	0.9	12.1
Seed width	0.0	0.1	6.0	7.9	2.2	57.8	0.4	9.4
Seed thickness	0.0	0.0	6.8	9.9	4.0	73.6	0.9	12.0
Seed weight with wing	1.2	1.2	17.2	17.3	5.1	98.3	2.2	35.2
Seed weight without wing	1.2	1.2	18.2	18.3	2.3	98.3	2.2	37.2
Seed moisture (%)	1.6	14.4	30.6	32.5	11.1	11.5	0.3	7.7
Germination (%)	239.6	290.3	25.6	28.2	11.8	98.8	28.9	115.1
Germination value	0.0	0.0	55.9	57.1	30.9	90.0	2.1	48.0
Peak value	0.0	0.0	39.0	43.2	18.6	81.4	0.1	72.0
Mean daily germination	0.0	0.0	25.4	28.4	12.7	80.0	0.2	46.9
Mean germination time	1.6	2.3	21.6	25.9	4.9	67.6	0.0	36.7
Germination speed	0.1	0.1	36.5	39.3	14.5	86.3	0.6	70.0
Germination energy index	41.5	50.4	25.6	28.2	11.8	82.5	12.0	48.0
Plumule length	1.6	1.6	48.4	48.7	5.3	82.5	2.6	99.1
Radicle length	0.3	0.3	30.9	31.4	5.3	97.0	1.1	62.8
Seedling length	3.4	3.4	40.8	41.1	15.0	98.5	3.7	83.6
Plumule-radicle ratio	0.1	0.1	24.8	26.3	7.2	92.1	0.6	49.2

The findings suggested that the differences in cone features between seed sources are probably due to different levels of natural constraints in the locations, where the seeds were originally found. Most physical characteristics of cone and seed are mostly determined by the genotype of individual trees. Similarly, Burdon and Lou, 1973 found that the cone trait of *Pinus radiata* was under genetic control. The traits of these species are interdependent and mostly genetically determined features. The current study revealed that cone weight showed highest in both phenotypic and genotypic variance as well as phenotypic and genotypic coefficient of variance than rest of the cone parameters. Results also showed that the phenotypic coefficient of variance of cone weight was lower than genotypic coefficient of variance, however as a whole difference was quite small, which suggests that cone weight is controlled by the environment as well as genetic components (Meena *et al.,* 2015). The length, width, thickness and weight of seeds are also controlled by the environment as well as genetic components. Both internal (maternal and genetic) and exterior (environmental) factors may have contributed to the difference in seed size at the time of seed development in a variety of environments (Harper *et al.*, 1970). Overall, the plants populations within an area have influence on location-specific environmental factors (Khalil, 1986).

The significant variance, variability and genetic components were observed in germination traits. The germination % showed highest phenotypic and genotypic variance; whereas, germination value showed highest phenotypic, genotypic and environment coefficient of variance. The genotypic variance may be due to the locations of different seed sources. Also, genotypic coefficient of variance is more in all germination traits than phenotypic coefficient of variance which indicates that a trait is controlled by genetic as well as environment component (Dobhal *et al.*, 2019b). Furthermore, when trees are grown in the different environment then, seedlings from diverse seed sources frequently show distinct patterns of shoot growth (Soresen, 1979). In the current study, when seed of all seed sources are raised under common nursery conditions, hence the variation thus obtained is genetic in nature, whether the provenance is of outside and inside nursery (Sneizko and Stewart, 1989). Moreover, the parameters governing seed germination & seedling growth are interrelated and are depended by the genetic composition of the seed, environmental factors and seed characteristics (Pathak *et al.*, 1984).

Correlation coefficient of different parameters with altitude, latitude and longitude of seed sources

Correlation coefficient between altitude, latitude and longitude was worked out with cone, seed and seedling traits of *P. wallichiana*. Altitude showed significant ($p \leq 0.01$) positive correlation with seed length with wing (0.79) and without wing (0.84), 100 seed weight with wing (0.83), 100 seed weight without wing (0.82), germination % (0.86), germination value (0.79), daily germination (0.86), germination speed (0.78), germination energy index (0.86), plumule length (0.91), radicle length (0.88), seedling length (0.91) and plumule-radicle ratio (0.76); whereas, at $p \leq 0.05$ positive correlation was recorded for scale/cone (0.71), seed thickness (0.72), peak value (0.75) and germination time (0.73). Latitude showed significant ($p \leq 0.05$) positive correlation with only germination time (0.70) and radicle length (0.66) parameters. All the cone, seed and seedling characters did not exhibit any significant correlation with altitude (Table 7). Khalil, 1986, showed that non-significant of cone parameters trends of *Picea glauca*. Hussain, (2002) also reported that the same for *Cedrus deodara* in terms of weight and length of cones.

Journal of Genetics, Genomics & Plant Breeding 9(3) 84-100 (July, 2025)
ISSN (Online): 2581-3293 DOI: 10.63551/jggpb.vol.9.issue03.003

Table 7: Correlation coefficient of different parameters with altitude, latitude and longitude of seed sources

Parameters	Altitude	Latitude	Longitude
Cone length	0.47^{NS}	0.37^{NS}	-0.33^{NS}
Cone diameter	0.4^{NS}	0.58^{NS}	-0.03^{NS}
Cone weight	0.62^{NS}	0.47^{NS}	-0.25^{NS}
Scale/cone	0.71^{*}	0.52^{NS}	-0.37^{NS}
Seed/cone	0.44^{NS}	0.29^{NS}	0.22^{NS}
Seed length with wing	0.79^{**}	0.53^{NS}	-0.10^{NS}
Seed length without wing	0.84^{**}	0.44^{NS}	-0.07^{NS}
Seed width	0.57^{NS}	-0.11^{NS}	-0.22^{NS}
Seed thickness	0.72^{*}	0.22^{NS}	-0.07^{NS}
Seed weight with wing	0.83^{**}	0.45^{NS}	0.07^{NS}
Seed weight without wing	0.82^{**}	0.44^{NS}	0.10^{NS}
Seed moisture (%)	0.23^{NS}	0.23^{NS}	0.13^{NS}
Germination (%)	0.86^{**}	0.49^{NS}	-0.03^{NS}
Germination value	0.79^{**}	0.28^{NS}	0.08^{NS}
Peak value	0.75^{*}	0.18^{NS}	0.07^{NS}
Mean daily germination	0.86^{**}	0.49^{NS}	-0.02^{NS}
Mean germination time	0.73^{*}	0.70^{*}	-0.05^{NS}
Germination speed	0.78^{**}	0.18^{NS}	0.05^{NS}
Germination energy index	0.86^{**}	0.49^{NS}	0.03^{NS}
Plumule length	0.91^{**}	0.55^{NS}	0.04^{NS}
Radicle length	0.88^{**}	0.66^{*}	-0.13^{NS}
Seedling length	0.91^{**}	0.59^{NS}	-0.01^{NS}
Plumule-radicle ratio	0.76^{**}	0.27^{NS}	0.27^{NS}

*significant at<0.05,**significant at<0.01, NS-Non-significant

Elevated precipitation results in the leaching of nutrients, causing trees to develop slowly and producing smaller fruits and cones (Tripathi and Banik, 2001). Similar response in cone characteristics with respect to *Pinus roxburghii* was obtained by Roy *et al.*, (2004) and in *Pinus strobus* by Dermeritt and Hocker, (1975). Kumar and Banerjee, 1986 also showed that the altitude-wise variation in the cone size and weight and size of seeds that untimely affects the germination performance of seed sources. Altitudinal variation for seed weight has been stated by Isik, 1986 in *P. brutia* provenances. Birot (1978) reported that the identical relationship in 1000 seed weight of *Pseudostsuga menzissi* provenances. Also, the result is harmony with the formerly observation of Roy *et al.,* (2004) in *Pinus roxburghii*, Singh and Bhatt, (2008a) in *Dalbergia sissoo* and Saklani *et al.,* (2012) in *Quercus leucotrichophora.*

Correlation coefficient for various characteristics of *Pinus wallichiana*

Correlation coefficient (Karl Pearson's) was worked out for 23 characters of cone, seed and seedling of *P. wallichiana*. Among the different parameters being studied, 102 correlation were found positive significant at $p \leq 0.01$ and 60 correlation were found positive significant at $p \leq 0.05$. Significant correlation at $p \leq 0.01$ were found between traits of cone with other important traits like cone length which is positively correlated with cone diameter (0.88) and seed/cone (0.83), cone diameter which is positively correlated with scale/cone (0.81) and daily germination (0.82); whereas, cone weight which is positively correlated with seed/cone (0.93). Significant correlation at $p \leq 0.01$ were found between traits of seed with other important traits like seed length with wing and without wing, seed thickness and seed weight with wing and without wing which is positively correlated with seed moisture %, peak value, germination speed, germination energy index, radicale length and plumule radicle ratio. Significant correlation at $p \leq 0.01$ were found between germination traits with other important traits like germination %, germination and peak value, daily germination, germination time & their speed and germination energy index which is positively correlated with radicale length and seedling length. Significant correlation at $p \leq 0.01$ were found between length of plumule and radicale which is positively correlated plumule radicale ratio; whereas seedling length and plumule radicale ratio, show significant correlation among themselves (Table 8). At the same time, significant correlation at $p \leq 0.05$ were found between cone diameter and weight, scale/cone and seed/cone which is positively correlated with seed moisture %, peak value and germination speed; whereas, seed length with wing and without wing, seed thickness and seed weight with wing and without wing which is positively correlated with daily germination only. In case of germination and growth parameters, germination speed which is positively correlated with plumule length (0.71); whereas, radicale length which is positively correlated with seedling length (0.65), and seedling length which is positively correlated with plumule-radicle ratio (0.76) (Table 8).

Kumar and Banerjee, 1986 also reported that the substantial relationship between *A. nilotica* seed weight and germination homogeneity. Significant correlation of length and weight of seed with germination percentage and germination value in *P. roxburghii* was reported by Mukherjee (2005). This relationship may be due to the inter dependence of all the parameters. Similar finding was observed by Rawat and Bakshi, (2011) in *Pinus wallichiana*, Singh *et al.*, (2015) in *Pinus kesiya* and Chauhan and Kanwar, (2001) in *Pinus roxburghii*.

In conclusion the cone, seed and seedling parameters of *Pinus wallichiana* in terms of genetic parameters with variety of seed sources showed a broad range of variability. Significant correlations were noted between the majority geographical parameters with specifically altitude. The results of the studies showed that breeding and improvement methods can be achieved by selecting certain key traits. During the course of investigation, it is concluded that Gangotri seed source showed desirable results in the relation to seed morphological, germination with their growth parameters. Such an analysis could aid in the selection of superior provenance/seed source for a particular site in order to design strategies for the species' improvement, breeding and conservation.

Journal of Genetics, Genomics & Plant Breeding **9(3) 84-100 (July, 2025)**
ISSN (Online): 2581-3293 DOI: 10.63551/jggpb.vol.9.issue03.003

Table 8. Correlation coefficient between various cone, seed and seedling characters of *Pinus wallichiana*

Parameters	Cone length	Cone diameter	Cone weight	Scale/cone	Seed/cone	Seed length with wing	Seed length without wing	Seed width	Seed thickness	Seed weight with wing	Seed weight without wing	Seed moisture (%)	Germination (%)	Germination value	Peak value	Mean daily germination	Mean germination time	Germination speed	Germination energy index	Plumule length	Radicle length	Seedling length	Plumule-radicle ratio
1	0.5																						
2	0.8**	0.6*																					
3	0.5	0.5	0.7*																				
4	0.6*	0.8**	0.5	0.4																			
5	0.8**	0.6	0.9**	0.7*	0.6*																		
6	0.3	0.5	0.6*	0.7*	0.4	0.7**																	
7	0.3	0.2	0.3	0.6*	0.3	0.4	0.4																
8	0.2	0.4	0.3	0.3	0.4	0.4	0.7*	0.4															
9	0.2	0.5	0.4	0.5	0.3	0.5	0.7*	0.1	0.6*														
10	0.2	0.4	0.4	0.5	0.3	0.5	0.7*	0.1	0.6*	0.9**													
11	-0.2	-0.0	0.0	-0.0	-0.2	0.0	0.3	-0.4	0.1	0.6*	0.6*												
12	0.5	0.7*	0.7*	0.7*	0.6*	0.8**	0.8**	0.4	0.8**	0.8**	0.8**	0.2											
13	0.6	0.47	0.7*	0.6	0.6	0.8**	0.8**	0.3	0.6*	0.6*	0.6*	0.3	0.9**										
14	0.6	0.35	0.7*	0.5	0.5	0.8**	0.7**	0.3	0.5	0.6	0.6	0.3	0.8**	0.9**									
15	0.5	0.70*	0.7*	0.7*	0.6*	0.8**	0.8**	0.3	0.8**	0.8**	0.8**	0.3	0.9**	0.9**	0.8**								
16	0.3	0.82**	0.4	0.6	0.6*	0.62*	0.7*	0.2	0.7*	0.7*	0.7*	0.1	0.8**	0.5	0.4	0.8**							
17	0.5	0.50	0.7*	0.6	0.6	0.78**	0.8**	0.4	0.7*	0.7*	0.6*	0.3	0.9**	0.9**	0.9**	0.9**	0.5**						
18	0.5	0.7*	0.7*	0.7*	0.6*	0.8**	0.8**	0.4	0.8**	0.8**	0.8**	0.2	1.0**	0.9**	0.8**	0.9**	0.8**	0.9**					
19	0.4	0.5	0.6*	0.6*	0.5	0.8**	0.9**	0.3	0.7**	0.8**	0.8**	0.3	0.9	0.8	0.8	0.9	0.8	0.8	0.9				
20	0.5	0.4	0.6*	0.6*	0.4	0.8**	0.8**	0.3	0.7*	0.7*	0.0*	0.2	0.8**	0.7**	0.7*	0.8**	0.7**	0.7*	0.8**	0.9**			
21	0.5	0.5	0.6*	0.7*	0.5	0.8**	0.9**	0.3	0.7**	0.7**	0.7**	0.3	0.9**	0.8**	0.7**	0.9**	0.8**	0.8**	0.9**	0.9**	0.9**		
22	0.4	0.7*	0.5	0.5	0.7*	0.6*	0.8**	0.3	0.7*	0.8**	0.7**	0.2	0.9**	0.8**	0.7**	0.9**	0.7**	0.8**	0.9**	0.8**	0.6*	0.7**	
23	0.4*	0.4*	0.1	0.6**	0.7**	0.7**	0.7**	0.7	0.8**	0.6**	0.7**	0.8	0.8**	0.9**	0.2	0.9**	0.9**	0.8**	0.8**	0.6**	0.8**	0.7*	0.7**

1Cone Length,2Cone diameter,3Cone weight,4Scale/cone,5Seed/cone,6Seed length with wing,7Seed length without wing,8Seed width,9Seed thickness,10Seed weight with wing,11Seed weight without wing,12Seed moisture (%),13Germination (%),14Germination value,15Peak value,16Mean daily germination,17Mean germination time,18Germination speed,19Germination energy index,20Plumule length,21Radicle length,22Seedling length,23Plumule-radicle ratio *significant at <0.05,** significant at<0.01

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