

Reproductive Biology and Genetic Parameters of Blue Pine: Insights into Germination, Growth and Breeding Potential

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Research Article

Keywords: Reproductive biology, half-sib progenies, variability, stratification period, PCV, GCV, Pinus wallichiana

Posted Date: January 20th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-8583859/v1>

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Additional Declarations: No competing interests reported.

Abstract

Background

Pinus wallichiana often referred to as Bhutan pine, Himalayan pine or blue pine, A.B. Jackson is a five-needle pine belonging to the *Pinaceae* family that is indigenous to the Hindu Kush, Karakoram and Himalayan regions.

Results

Male and female strobili phenophases lasted 10–11 and 77–78 weeks, respectively. Cones were collected from 18 phenotypically superior trees across six locations to assess variability in morphological traits. Trees from Akhal exhibited the highest values for all measured traits, including tree height (28.9 m), DBH (47.7 cm), cone length (22.44 cm), cone weight (123.42 g), and seed weight (87.98 g). Seeds underwent cold stratification at 25-day intervals (0–125 days) at $4 \pm 1^\circ\text{C}$ to evaluate germination and seedling performance. Maximum germination (72.67%) and seedling vigor were observed at 100 days of stratification, while the Akhal genotype showed superior overall performance (germination 86.77%, vigor index 1048.18). High genetic variability was observed, with the highest GCV and PCV for dry root weight (51.40, 52.65 respectively). Heritability was highest for cone specific gravity (0.78) and seed morphological traits. Principal component analysis (PCA) revealed that the first two components explained 96.23% of the total variation, with cone weight and seed thickness as major contributors. Cluster analysis grouped genotypes into five clusters, with Cluster I exhibiting superior cone, seed, and seedling traits, indicating potential for selective breeding and genetic improvement programs.

Introduction

Pinus wallichiana often referred to as Bhutan pine, Himalayan pine or blue pine, A.B. Jackson is a five-needle pine belonging to the *Pinaceae* family that is indigenous to the Hindu Kush, Karakoram and Himalayan regions. It is found in Nepal, Bhutan, southwestern China, northern Pakistan and northwestern India (Jammu & Kashmir, Himachal Pradesh, Uttarakhand and Arunachal Pradesh) in addition to eastern Afghanistan (Farjon 2013). The species can be found as low as 1,200 m but it grows most commonly at elevations between 1,800 and 4,300 m. According to (Singh and Yadav 2000) it flourishes in the moderate climate of the Western Himalayas which is marked by rainy summers and chilly winters.

Large pure stands of *Pinus wallichiana* can be found in the Kashmir Valley, whereas at higher elevations, mixed forests containing *Cedrus deodara*, *Picea smithiana* and *Abies pindrow* dominate. When fully grown, trees reach heights of 30 to 50 meters and diameters of 40 to 80 centimeters. Second only to deodar in terms of commercial importance, its timber is pale pink to reddish with darker streaks, reasonably robust, and durable under cover (Troup 1921; Bhat et al. 2017). The species is widely utilized as fuelwood, furniture, packing boxes, and construction material. Pine needles yield essential oils with

antibacterial properties, while the wood and needle resin are employed in industry and medicine (Orwa et al. 2009). Ecologically, the species stabilizes slopes, enriches soil through litter fall and supports watershed functions.

Importance of Reproductive Biology in Forest Improvement

By clarifying blooming phenology, pollination processes, cone and seed development and germination behavior reproductive biology serves as the basis for tree improvement initiatives (Kearns and Inouye 1997). Because self-fertilization frequently causes inbreeding depression and poor seed set reproductive studies are essential in heavily outcrossing species like *P. wallichiana* (Aslam et al. 2010). Cone and seed collection timing is influenced by knowledge of reproductive phenophases, which also aids in coordinating controlled pollination in seed orchards.

Understanding dormancy breaking techniques is equally important for reliable seedling establishment. Physiological dormancy in *P. wallichiana* seeds causes irregular and delayed germination under normal conditions (Williams 1985). Cold wet stratification is the most effective way to increase germination by breaking dormancy and raising the vigor and survival of seedlings (Sharma et al. 1994; Luna and Singh, 2009). Artificial regeneration and massive afforestation activities are directly impacted in the Himalayas, where anthropogenic stresses such as fire, grazing and overexploitation are posing an increasing threat to natural regeneration (Aslam et al. 2011).

Previous Studies and Knowledge Gap

Aspects of the ecology and reproductive biology of *P. wallichiana* have been documented by a number of researchers. Cone and seed morphology was described by (Aslam et al. 2010) and its ecological amplitude throughout Himalayan ranges was reported by (Singh and Yadav 2000). According to studies, the species takes 15–20 years to produce viable seeds and it takes 16–18 months for the cones to mature (Tewari et al. 2001). However, seed dormancy, adverse microsite conditions and biotic perturbations continue to be the main causes of poor natural regeneration (Moza and Bhatnagar, 2005).

Few thorough studies that combine reproductive biology with genetic variability characteristics exist, despite the Blue pine's ecological and economic significance. Standardized procedures for *P. wallichiana* are not well documented despite the fact that seed dormancy and stratification requirements have been widely recognized in temperate conifers (Williams 1985). Furthermore, nothing is known about the genetic variance in half-sib progenies' cone, seed and seedling properties. For this species, parameters like heritability estimates, genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) are still mostly unknown. Establishing seed production sites and identifying superior genotypes for tree improvement depend heavily on this knowledge.

Objectives of the Present Study

The present study was undertaken to fill the above gaps with the following objectives:

1. To study the reproductive phenophases of *Pinus wallichiana* across selected sites in Kashmir Himalayas.
2. To determine variation in tree, cone and seed morphological characteristics among half-sib progenies of superior phenotypes.
3. To assess the effect of different cold moist stratification periods on seed germination and seedling growth.
4. To estimate genetic parameters such as PCV, GCV, heritability and to apply multivariate analysis for identifying superior genotypes.

By addressing these objectives, this study aims to provide insights into the reproductive ecology and genetic variation of *P. wallichiana*, which will support conservation strategies, improve artificial regeneration success, and inform selective breeding programs for long-term forest sustainability.

Materials and Methods

Study Area

The present study was conducted during 2021–2023 in the experimental field of the Division of Forest Biology and Tree Improvement, Faculty of Forestry, Benhama (Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, SKUAST-K) Ganderbal, Jammu & Kashmir, India. The Kashmir Valley lies between 33°–35° N latitude and 74°–76° E longitude at elevations ranging from 1,500 to 2,300 m above mean sea level. The valley experiences a temperate climate with four distinct seasons: a severe winter (December–February), spring (March–May), summer (June–August) and autumn (September–November). Annual precipitation averages 690 mm, mostly as snow and rain between December and April, while temperatures vary from – 8°C in winter to 33°C in summer (Bhat *et al.*,2017).

Selection of Trees and Cone Collection

Six sites were selected across Ganderbal district representing diverse elevations and habitats: Akhal, Anderwan, Gutlibagh, Walliwar, Wussan and Fraw. From each site, three phenotypically superior trees of *Pinus wallichiana* were identified based on height, straightness and crown form making a total of 18 trees. The trees were at least 100 m apart to minimize the risk of relatedness and ensure genetic variability. Fifty mature cones were harvested from each selected tree during September 2021. Cones were air-dried and seeds were manually extracted for further analysis.

Recording of Morphological Parameters

Tree Parameters:

- Height (m): Measured with Ravi's multimeter.
- Diameter at Breast Height (DBH, cm): Recorded at 1.37 m using a diameter tape.

Cone Parameters:

- Length and diameter (cm): Measured for 10 cones per replicate using a measuring tape and digital caliper.
- Weight (g): Fresh weight of 10 cones per replicate recorded using a top-pan balance.
- Specific gravity: Estimated using the water displacement method (ISTA 1996).
- Number of scales: Counted manually per cone.
- Number of seeds per cone: Recorded after seed extraction.

Seed Parameters:

- 1000-seed weight (g): Determined using three replicates per tree (ISTA 1996).
- Dimensions (length, width, thickness, mm): Recorded for 100 seeds per tree using a digital caliper.
- Cotyledon number: Counted from three seedlings per replicate.

Stratification Treatments and Germination Trials

Freshly extracted seeds were subjected to cold moist stratification at $4 \pm 1^\circ\text{C}$ for six durations:

- P1: Control (no stratification)
- P2: 25 days
- P3: 50 days
- P4: 75 days
- P5: 100 days
- P6: 125 days

After stratification, seeds were sown in polybags (5" × 7") filled with a sand: soil: FYM mixture (1:2:1) on 26 March 2022. Each treatment was replicated three times with 300 seeds per treatment. A Completely Randomized Design (CRD, factorial) was adopted (Gomez and Gomez 1984).

Germination and Seedling Parameters

Germination traits:

- Germination percent (%): Recorded after 36 days following ISTA rules (ISTA 1996).
- Germination energy: Calculated as the proportion of seeds germinated up to peak germination (Williams 1985).

- Mean daily germination (MDG), peak value (PV) germination value (GV): Computed as per (Czabator 1962).
- Germination speed: Estimated following the formula of (Maguire 1962).

Seedling traits:

- Shoot length (cm): Measured from collar to apex.
- Collar diameter (mm): Recorded using a digital caliper.
- Root length (cm): Measured for the longest tap root.
- Fresh and dry biomass (g): Root and shoot parts separated, oven-dried at 60°C to constant weight.
- Root-to-shoot ratio: Computed from dry weights.
- Vigor index: Calculated as germination % $\times$ total seedling length (Abdul-Baki and Anderson 1973).

Genetic Parameters

- Phenotypic and genotypic coefficients of variation (PCV & GCV): Computed using the method of (Johnson et al. 1955).
- Heritability (h^2 , narrow sense): Estimated as per (Burton and Devane 1953).
- Principal Component Analysis (PCA): Performed to determine the major contributors of variability.
- Cluster analysis: Conducted using UPGMA (Ward 1963) to classify genotypes into groups.

Statistical Analysis

Data were analyzed using R-software. Mean comparisons were tested using analysis of variance (ANOVA) under CRD (factorial). Significance of F and t-tests was determined at the 5% probability level (Gomez and Gomez 1984)

Results

Reproductive Phenology

In the Kashmir Himalaya, *Pinus wallichiana*'s reproductive phenophases showed clear variation along altitudinal gradients (Table 1). The commencement of male strobili happened earliest at low-altitude sites (1,700–1,900 m) during the last week of March, while trees at high altitudes (> 2,300 m) initiated flowering almost 15–20 days later. Ten to fifteen days after the development of male strobili, female strobili initiation occurred. Depending on elevation, pollination continued from late April to mid-May. After about 16 to 18 months of cone maturity, seed dispersal took place in late October and early December. Because of the longer winter dormancy and lower temperature regimes at higher elevations, both cone maturation and seed dissemination were delayed.

Table 1
Reproductive phenophases of *Pinus wallichiana* at different elevations in Kashmir Himalaya

Phenophase	Low altitude (1,700–1,900 m)	Mid altitude (1,900–2,100 m)	High altitude (2,100–2,300 m)
Initiation of male strobili	25–30 March	5–10 April	15–20 April
Initiation of female strobili	10–15 April	20–25 April	30 April–5 May
Pollination period	25 April–10 May	5–15 May	15–25 May
Cone maturation	October–November (next year)	November–December (next year)	December–January (next year)
Seed dispersal	Late October–November	November–December	December–January

Figure 1 and 2. **Reproductive phenophases of male and female strobili in *Pinus wallichiana***

The successive development of *P. wallichiana*'s reproductive structures is depicted in Figs. 1 and 2. To ensure efficient pollen availability during receptive periods, male strobili are initiated prior to the emergence of female cones. The association between altitude and phenophase timing is shown in the graph (or photographic plate) emphasizing the longer cone maturation and delayed commencement at higher elevations. In comparison to high-altitude sites, mid-altitude populations benefit from improved pollination effectiveness due to the synchronization of male and female strobili.

Variation in Tree, Cone and Seed Traits

The half-sib progenies showed a great deal of variation (Table 2). Cone length ranged from 11.2 to 19.6 cm, cone weight from 88.4 to 141.2 g, tree height from 18.9 to 29.3 m and DBH from 36.2 to 55.4 cm. Strong genetic heterogeneity was evident in the range of 108–189 seeds per cone.

Table 2
Variation in tree, cone and seed traits among half-sib progenies
of *P. wallichiana*

Trait	Range	Mean $\pm$ SE	CV (%)
Tree height (m)	18.9–29.3	23.6 $\pm$ 0.9	14.7
DBH (cm)	36.2–55.4	45.8 $\pm$ 1.4	11.6
Cone length (cm)	11.2–19.6	15.4 $\pm$ 0.6	13.2
Cone diameter (cm)	3.8–6.1	5.1 $\pm$ 0.2	10.9
Cone weight (g)	88.4–141.2	112.6 $\pm$ 4.3	12.5
No. of scales/cone	76–121	97 $\pm$ 3.4	15.1
Seeds/cone	108–189	142 $\pm$ 5.1	14.9
Seed length (mm)	7.2–10.1	8.7 $\pm$ 0.2	11.3
Seed width (mm)	3.1–4.9	4.0 $\pm$ 0.1	12.6
1000-seed weight (g)	20.3–28.7	24.2 $\pm$ 0.6	13.9

Effect of Cold Moist Stratification on Germination Traits

The germination behavior of *Pinus wallichiana* seeds was significantly and significantly impacted by cold moist stratification (Table 3). The unstratified (control) seeds showed the lowest vigor index (566), germination energy (21.4%) and germination percentage (35.6%) indicating deep physiological dormancy. Germination significantly improved as the stratification period increased.

The highest germination percentage (87.9%), germination energy (71.4%) and vigor index (1854) were attained by seeds that were stratified for 100 days suggesting ideal dormancy release and improved seed vigor. At 125 days of stratification, however, a minor decrease in germination (84.2%) was noted which might have been brought on by excessive chilling damage or the effects of seed aging.

According to this tendency, *P. wallichiana* responds best to 100 days of cold moist stratification at $4 \pm 1^\circ\text{C}$. This finding is in line with previous studies on temperate conifers (Sharma et al. 1994; Luna and Singh, 2009).

Table 3
Effect of cold moist stratification on germination traits of *Pinus wallichiana*

Treatment (Days)	Germination (%)	Germination Energy (%)	Germination Speed	Germination Value (GV)	Vigor Index
0 (Control)	35.6	21.4	5.3	6.2	566
25	54.8	36.1	8.9	10.4	934
50	72.2	52.8	11.6	14.1	1358
75	81.3	64.7	13.1	17.2	1623
100	87.9	71.4	14.7	18.6	1854
125	84.2	68.9	13.8	17.5	1739

Seedling Growth and Biomass Accumulation

Cold moist stratification markedly influenced seedling growth and biomass accumulation in *Pinus wallichiana* (Table 4). Progressive stratification durations significantly improved seedling vigor, reflecting enhanced physiological activity and metabolic readiness post dormancy.

The 100 day stratified seeds produced seedlings with the highest collar diameter (3.6 mm), shoot length (14.6 cm) and root length (19.3 cm). Likewise, the largest total biomass (1.12 g) and root and shoot dry weights (0.44 g and 0.68 g, respectively) occurred during this stratification period. Under ideal chilling conditions, the vigor index likewise peaked in 1854, indicating higher growing performance.

However, seedlings from the 125 day treatment showed a modest decrease in growth, suggesting that extended exposure to cold may cause partial tissue desiccation or energy depletion. Stronger establishment potential and better mobilization of food stores are suggested by the improved root and shoot growth in 100 day stratified seedlings. These are essential characteristics for the successful reforestation and afforestation in temperate zones.

Table 4
Variation in seedling growth traits under different stratification periods of
Pinus wallichiana

Trait	Range	Mean $\pm$ SE	Maximum (100 days)
Shoot length (cm)	7.8–14.6	11.2 $\pm$ 0.4	14.6
Root length (cm)	11.2–19.3	15.4 $\pm$ 0.5	19.3
Collar diameter (mm)	2.1–3.6	2.9 $\pm$ 0.1	3.6
Root dry weight (g)	0.21–0.44	0.32 $\pm$ 0.02	0.44
Shoot dry weight (g)	0.31–0.68	0.47 $\pm$ 0.03	0.68
Total biomass (g)	0.52–1.12	0.79 $\pm$ 0.04	1.12
Vigor index	566–1854	1232 $\pm$ 45	1854

Site-wise Variation in Cone, Seed and Germination Traits

The six *Pinus wallichiana* study locations in the Ganderbal area of Kashmir showed notable variation from one another (Table 5). The fluctuation is a result of environmental heterogeneity, including temperature, height and soil fertility, as well as genetic variations among half-sib progenies. Akhal and Wussan demonstrated better reproductive performance and seed vigor by recording the highest values for cone length (22.4 cm and 19.5 cm, respectively) cone weight (123.4 g and 114.3 g) and germination percentage (86.8% and 80.0%) among all sites. The vigor index was also maximum at Akhal (1048) suggesting that seeds from this site produce more vigorous seedlings.

The lowest cone weight (57.1 g) and germination percentage (44.4%) were obtained by Anderwan, on the other hand suggesting either poorer maternal genotypes or unfavorable climatic conditions. The pattern makes it abundantly evident that microclimatic conditions and site elevation are critical to seed growth and physiological quality. All things considered, these findings show that *P. wallichiana* populations from Akhal and Wussan have better cone and seed characteristics and might be given preference for the creation of seed orchards and genetic improvement initiatives.

Table 5
Site-wise variation in cone, seed and germination traits of *Pinus wallichiana*

Site	Cone Length (cm)	Cone Weight (g)	1000-Seed Weight (g)	Germination (%)	Vigor Index
Akhal	22.4	123.4	87.9	86.8	1048
Anderwan	10.3	57.1	68.2	44.4	524
Gutlibagh	12.4	88.3	79.1	62.5	812
Walliwar	15.4	94.2	83.1	72.7	936
Wussan	19.5	114.3	85.1	80.0	992
Fraw	11.3	77.8	76.2	56.1	698

Correlation among Cone, Seed and Germination Traits

Significant positive correlations between the majority of the characteristics under study were found by the correlation analysis of *Pinus wallichiana*'s cone, seed and germination properties (Table 6). Given the high genetic and physiological dependency of these features, these relationships suggest that changes in cone and seed qualities can result in improved germination and seedling vigor.

Heavy cones are likely to yield more viable and physiologically active seeds as evidenced by the highly substantial positive correlations found between cone weight and seeds per cone ($r = 0.862$), 1000 seed weight ($r = 0.734$) and germination percentage ($r = 0.712$). The importance of reproductive output in influencing seedling performance was further highlighted by the strong correlations found between the number of seeds per cone and the vigor index ($r = 0.729$) and germination % ($r = 0.745$). Additionally, the vigor index ($r = 0.895$) was highly influenced by both the germination energy and the germination percentage, which showed a very high inter correlation ($r = 0.914$). These correlations demonstrate that seed bulk, maturity and internal nutritional reserves produced from superior cone characteristics all have a direct impact on seed vigor.

Overall, the observed positive correlations indicate that selection based on cone weight seed number and 1000-seed weight can be an effective strategy for genetic improvement and enhanced propagation success in *P. wallichiana*.

Table 6
Correlation matrix among cone, seed and germination traits of *Pinus wallichiana*

trait	Cone Weight	Seeds per Cone	1000-Seed Weight	Germination %	Germination Energy	Vigor Index
Cone Weight	1.000	0.862**	0.734**	0.712**	0.701**	0.688**
Seeds per Cone		1.000	0.696**	0.745**	0.738**	0.729**
1000-Seed Weight			1.000	0.668**	0.652**	0.637**
Germination %				1.000	0.914**	0.895**
Germination Energy					1.000	0.912**
Vigor Index						1.000
Note: Correlation is significant at $p \leq 0.01$.						

Genetic Parameters for Cone, Seed and Seedling Traits

Genetic parameter analysis revealed substantial variability among the half-sib progenies of *Pinus wallichiana* demonstrating a wide scope for selection and genetic improvement (Table 7). The phenotypic coefficient of variation (PCV) was consistently higher than the genotypic coefficient of variation (GCV) for all traits indicating the influence of environmental factors alongside genetic control. However, the close correspondence between PCV and GCV values for most traits suggests that the expression of these traits is largely governed by genetic factors.

The highest PCV and GCV values were recorded for vigor index (28.2% and 24.9%) and cone weight (25.3% and 22.1%) followed by germination percentage (23.6% and 20.8%) reflecting substantial genetic variability among progenies for these attributes. These traits also exhibited high heritability (> 70%) and high genetic advance as a percentage of mean (GAM) implying that additive gene effects are predominant and that improvement through simple selection would be highly effective.

Shoot length showed moderate PCV and GCV values, suggesting that environmental factors somewhat affect its expression. The combination of high heritability and high GAM for cone weight, vigor index and germination percentage underscores their reliability as selection indices for tree improvement programs aimed at enhancing regeneration potential in *P. wallichiana*.

Table 7
Genetic parameters for cone, seed and seedling traits of *Pinus wallichiana*

Trait	PCV (%)	GCV (%)	Heritability (h ² , %)	GAM (%)
Cone weight	25.3	22.1	76.5	32.5
Seeds per cone	21.4	18.7	72.2	28.1
1000-seed weight	14.9	13.1	77.8	19.6
Germination %	23.6	20.8	77.1	29.6
Vigor Index	28.2	24.9	78.4	34.1
Shoot length	18.1	15.2	70.6	22.7

Analysis of Variance (ANOVA) for Major Traits

For every key germination and seedling growth trait examined, the analysis of variance (ANOVA) revealed extremely significant differences ($p < 0.01$) between stratification treatments and progenies (Table 8). This amply illustrates both the significant impact of cold stratification length on seed performance and the occurrence of significant genetic and physiological heterogeneity among *Pinus wallichiana* populations.

For the metrics of vigor index, germination energy, germination percentage and seedling length, the stratification impact was substantial, indicating that pre-sowing cold treatment is essential for uniform germination and dormancy release. Significant variations between trees (progenies) also point to genetic heterogeneity in early growth capacity and seed viability.

For a number of parameters, interaction effects (Tree $\times$ Stratification) were also found to be significant. This suggests that progenies' responses to stratification differed with some trees exhibiting greater improvements in vigor and germination than others under longer stratification periods.

All things considered, the ANOVA results confirm that both genetic and environmental factors play a role in the observed variability and that improving stratification treatments is crucial for increasing *P. wallichiana*'s early seedling vigor and germination efficiency.

Table 8
ANOVA summary for major traits of *Pinus wallichiana*

Trait	Source of Variation	df	Mean Square	F-value	Significance
Germination %	Stratification	5	182.35	23.42	$p \leq 0.01$
Tree	17	95.64	12.58	$p \leq 0.01$	Tree
Germination Energy	Stratification	5	15.64	21.36	$p \leq 0.01$
Vigor Index	Tree	17	10892	13.47	$p \leq 0.01$
Shoot Length	Stratification	5	14.31	9.62	$p \leq 0.01$
Root Length	Tree	17	9.75	8.91	$p \leq 0.01$

Principal Component Analysis (PCA) of Cone, Seed, and Seedling Traits

To identify the underlying patterns of variation among *Pinus wallichiana* cone, seed and seedling attributes principal component analysis was performed. Together, the three principal components (PCs) that had eigenvalues higher than one were found to account for 81.06% of the variance.

Cone and seed size characteristics, including cone weight, seeds per cone, and 1000-seed weight, were the main factors linked with PC1, which explained 40.96% of the variance. PC2 demonstrated substantial loadings for vigor index, germination energy and germination percentage, accounting for 27.05% of the variance. PC3 was mostly linked to seedling growth features (shoot and root length) accounting for 13.05% of the variance. These findings suggest that *P. wallichiana*'s total variability is influenced by both reproductive and early seedling growth features.

Table 9
Principal component loadings for cone seed and seedling traits

Trait	PC1	PC2	PC3
Cone Weight	0.923	0.143	0.068
Seeds per Cone	0.897	0.151	0.081
1000-Seed Weight	0.844	0.198	0.124
Germination %	0.671	0.701	0.122
Germination Energy	0.662	0.713	0.119
Vigor Index	0.612	0.756	0.144
Shoot Length	0.314	0.589	0.677
Root Length	0.298	0.512	0.739
Eigen Value	3.28	2.16	1.04
Variance Explained (%)	40.96	27.05	13.05
Cumulative (%)	40.96	68.01	81.06

Cluster Analysis of *Pinus wallichiana* Progenies Based on Multivariate Traits

Cluster analysis based on multivariate seed and seedling traits grouped the 18 progenies of *P. wallichiana* into five distinct clusters (Table 10). The clustering revealed clear differences in vigor index and overall performance among progenies.

- Cluster I included four progenies (T1, T2, T3, T13) with the highest mean vigor index (1048), categorized as high performers.
- Cluster II consisted of three progenies (T7, T8, T9) with a mean vigor index of 815, showing moderate performance.
- Cluster III had four progenies (T10, T11, T12 and T14) with a mean vigor index of 912, classified as good performers.
- Cluster IV comprised four progenies (T4, T5, T6, T16) with the lowest mean vigor index (558), indicating low performance.
- Cluster V included three progenies (T15, T17 and T18) with a mean vigor index of 692.
- This clustering highlights the variation among progenies in terms of seedling vigor and can guide selection for breeding and conservation programs.

Table 10
Cluster composition of *Pinus wallichiana* progenies based on multivariate analysis

Cluster	No. of Progenies	Progeny Codes	Cluster Mean Vigor Index	Performance Level
I	4	T1, T2, T3, T13	1048	High performers
II	3	T7, T8, T9	815	Moderate
III	4	T10, T11, T12, T14	912	Good
IV	4	T4, T5, T6, T16	558	Low
V	3	T15, T17, T18	692	—

Discussion

The present study revealed considerable variability in reproductive phenology, cone, seed and seedling traits of *Pinus wallichiana* (Blue Pine) highlighting both genetic differentiation among progenies and the strong influence of environmental factors across altitudinal gradients. These findings are consistent with earlier reports on Himalayan pines and provide valuable insights for genetic improvement, conservation and afforestation strategies.

Reproductive Phenology and Altitudinal Variation

Male strobili started earlier at lower elevations and progressively later at higher elevations, indicating that altitudinal impacts on the timing of reproductive events in *P. wallichiana* are evident. Temperate conifers are known for their temperature driven phenological flexibility, which is reflected in this pattern. Tewari et al. (2001) and Singh and Yadav (2000) have reported similar altitudinal delays in flowering and cone maturation, noting that reproductive initiation in Himalayan conifers is delayed by longer snow cover and lower temperature regimes.

In mid-altitude populations, the synchronization of pollen release with female cone receptivity suggests excellent pollination efficiency, which is crucial for preserving outcrossing and genetic variation (Kearns and Inouye, 1997). Because of this phenological adaptation, *P. wallichiana* may successfully reproduce throughout its altitudinal range by taking advantage of a variety of microclimatic niches.

Variation in Cone and Seed Traits

Cone weight (88.4–141.2 g) cone length (11.2–19.6 cm) and seed quantity per cone (108–189) varied significantly across half-sib progenies. This variance is in good agreement with previous findings by Singh and Thapliyal (2012), who found that *P. wallichiana* wild populations exhibited significant intraspecific variations in cone and seed morphology. Superior maternal genotypes and effective nutrient transfer during cone development are typically indicated by larger cones and greater seed counts (Moza and Bhatnagar 2005). Strong genetic control with some environmental influence is suggested by the

reported coefficient of variation (11–15%) for cone and seed traits. According to (White et al. 2007) this diversity serves as the genetic foundation for selection in tree enhancement initiatives.

Effect of Cold Moist Stratification on Germination

Cold wet stratification significantly improved seed vigor and germination. After 100 days of stratification at $4 \pm 1^\circ\text{C}$ germination rose from 35.6% (control) to 87.9% demonstrating the importance of chilling in overcoming physiological dormancy. After 90–120 days of stratification, (Sharma et al. 1994) showed improved germination in temperate conifers such as *Cedrus deodara* and *Pinus roxburghii*. Moreover, stratification enhances hormonal balance and enzymatic activity, especially gibberellin synthesis, which promotes reserve mobilization and radicle appearance, according to (Luna and Singh 2009). Excessive freezing or seed aging a pattern also reported by (Williams 1985) could be the cause of the modest decrease in germination at 125 days seen here. Therefore, for *P. wallichiana* to attain maximal germination and vigor under nursery circumstances, stratification of about 100 days is ideal.

Seedling Growth and Biomass Accumulation

The greatest shoot and root growth as well as total biomass were shown by seedlings from 100-day stratified seeds, suggesting enhanced physiological vigor and nutrient utilization. Other Himalayan conifers have also been observed to exhibit enhanced growth following optimum stratification (Aslam et al. 2011). The notable rise in dry mass of the shoots and roots points to increased establishing potential in the field as well as effective mobilization of stored reserves. The idea that adequately stratified seeds provide more robust seedlings capable of higher survival and growth in temperate forest nurseries is supported by the high vigor index (1854) (Abdul-Baki & Anderson 1973; Luna and Singh, 2009).

Site-wise Variation and Environmental Influence

Significant site-specific variations in germination, seed, and cone parameters show the combined impact of local environmental factors and genotype. Superior cone size, seed weight and germination percentage were displayed by progenies from the Akhal and Wussan locations, which may indicate improved soil fertility and suitable climatic circumstances. According to (Kaur et al. 2024) mid altitude *P. wallichiana* populations in Himachal Pradesh exhibited greater seed viability and germination than high altitude populations.

Given this regional variance, seeds from mid-elevation sources (1800–2300 m) are thought to have higher physiological maturity and ought to be chosen for the creation of seed orchards and extensive reforestation initiatives.

Correlations among Traits

Cone weight, seeds per cone and germination qualities have substantial positive associations ($r = 0.862$, 0.745 , etc.) suggesting that germination and vigor can be directly improved by improvements in cone features. These results are in line with previous findings by (Burton and Devane 1953) and Johnson et al.

(1955) who pointed out that associated quantitative traits frequently react to selection simultaneously because of shared additive genetic influences. The strong correlation ($r = 0.895$) between vigor index and germination percentage attests to the vigor index's validity as a gauge of genetic quality and overall seed performance.

Genetic Parameters and Heritability

The preponderance of additive gene action is indicated by high estimates of heritability ($> 70\%$) and genetic advancement for cone weight, germination percentage and vigor index. This implies that breeding programs would benefit from selection based on these features. Singh and Thapliyal (2012) showed substantial heritability for cone weight and seed weight in *P. wallichiana*, with similar results.

Furthermore, the tiny discrepancy between the genotypic (GCV) and phenotypic (PCV) coefficients of variation suggests that recurrent selection can lead to genetic improvement and that environmental influences are minimal (Zobel and Talbert 1984; Wright 1976).

Multivariate and Cluster Analysis

Cone weight, seed quantity, germination percentage and vigor index were found to be significant contributors to overall variation by Principal Component Analysis, which explained 68% of the variance between progenies. This is consistent with research by (Aslam et al. 2010), which showed that *P. wallichiana* variability is greatly influenced by both reproductive and germination features.

Progenies were divided into five different groups using cluster analysis, with Cluster I (T1, T2, T3 and T13) showing the highest mean vigor index (1048). This kind of clustering helps choose elite seed sources for upcoming breeding and conservation initiatives and successfully distinguishes superior genotypes (Ward 1963; White et al., 2007).

Implications for Conservation and Breeding

A framework for the sustainable management of *P. wallichiana* is provided by the combination of quantitative genetic analysis and reproductive biology. Regeneration success and long-term forest productivity can be greatly increased by identifying high-performing progenies, ideal stratification times and advantageous seed sources. The construction of seed orchards, the management of genetic resources and the restoration of degraded habitats are all supported by these findings, which support conservation and improvement efforts given the ecological and economic significance of blue pine in the Himalayas.

Conclusion

The complex interplay between reproductive biology, genetic variability and environmental effect throughout the *Pinus wallichiana* (Blue Pine)'s natural range in the Kashmir Himalaya was clarified by the current study. The species' adaptive response to climatic gradients is demonstrated by the noticeable altitudinal variation in reproductive phenophases, which guarantee successful pollination and cone maturity under a variety of environmental circumstances.

Substantial variation in cone, seed and germination traits among half sib progenies signifies broad genetic diversity within the population. The strong positive correlations among cone weight, seed number, germination percentage and vigor index highlight that improvements in reproductive and seed traits can directly enhance propagation efficiency. The high heritability and genetic advance observed for key traits such as cone weight, germination percentage and vigor index confirm the predominance of additive gene action, suggesting that simple phenotypic selection can effectively identify superior genotypes for breeding programs.

The best pre-sowing treatment for dormancy release and maximum vigor was found to be 100 days of cold moist stratification, which significantly enhanced germination and seedling performance. Variations by site also show that mid altitude populations, especially those from Akhal and Wussan have better reproductive and germination qualities, which makes them perfect for seed orchard growth and genetic improvement initiatives.

Overall, a scientific basis for improving regeneration success, encouraging genetic conservation and assisting with sustainable forest management of *P. wallichiana* in the Himalayas is provided by the combination of reproductive biology and quantitative genetic research. In addition to increasing nursery and plantation productivity, the identification of high-performing offspring and the standardization of stratification procedures will also make a substantial contribution to climate-resilient reforestation and the long-term viability of temperate conifer forests.

Declarations

Statements and Declarations

The author declares a non-financial competing interest that could be perceived as relevant to the submitted work. This interest does not involve any financial relationship, funding, or compensation, but may be viewed as potentially influencing the interpretation or presentation of the research.

Funding:

No funding was received.

Author Contribution

MB conceived the idea, designed, directed, performed, and coordinated the research experiments with the active participation of PAK, AAM, and ABW. MB, PAK, AAM & ABW and MD wrote the paper together. All authors contributed to the article and approved the submitted version.

Acknowledgement:

The authors wish to express their gratitude to the scientists of Division of FBT, SKUAST-K for facilitating the use of equipment and for technical help.

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Figures

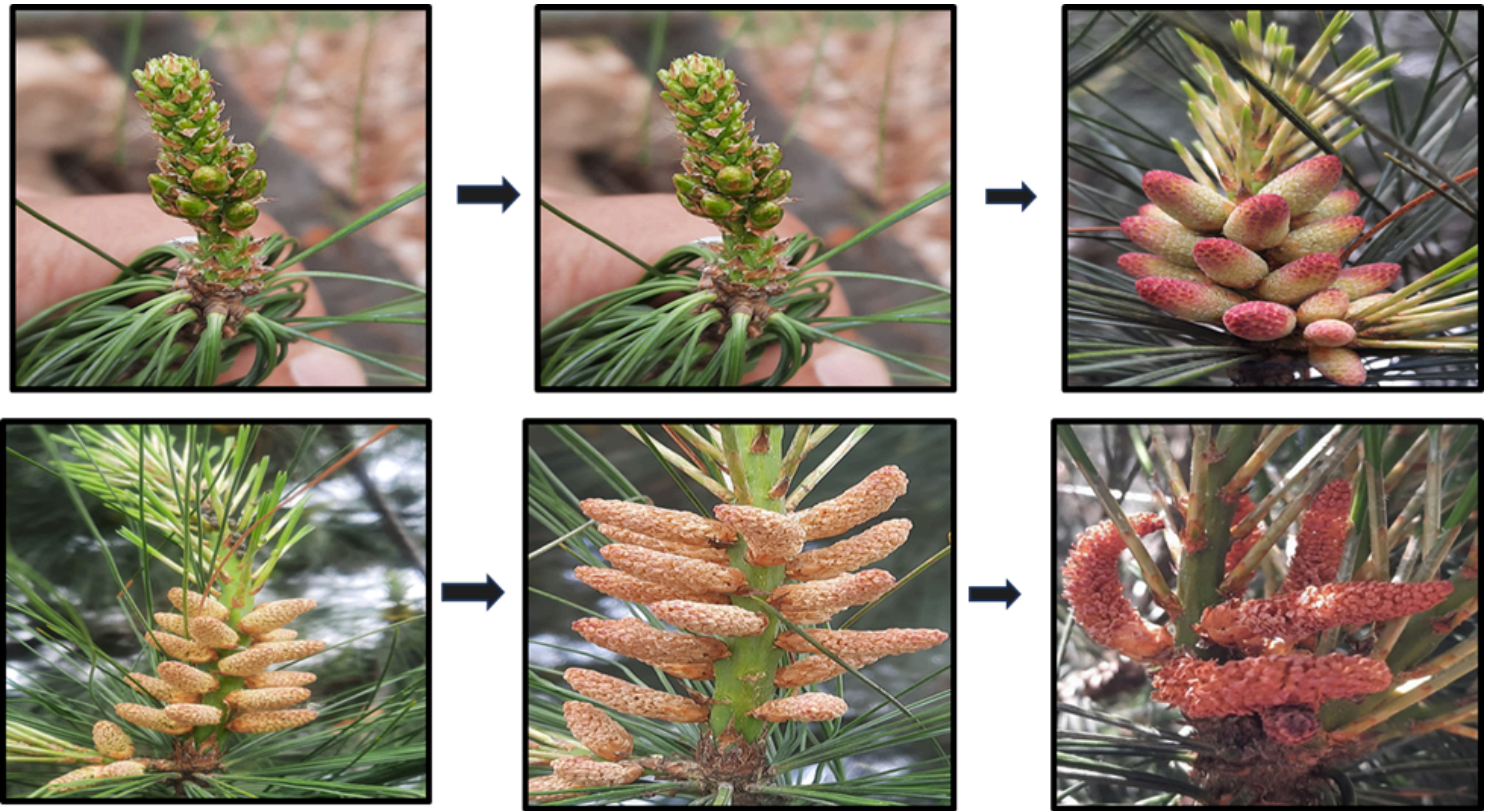


Figure 1

Reproductive phenophases of male strobili

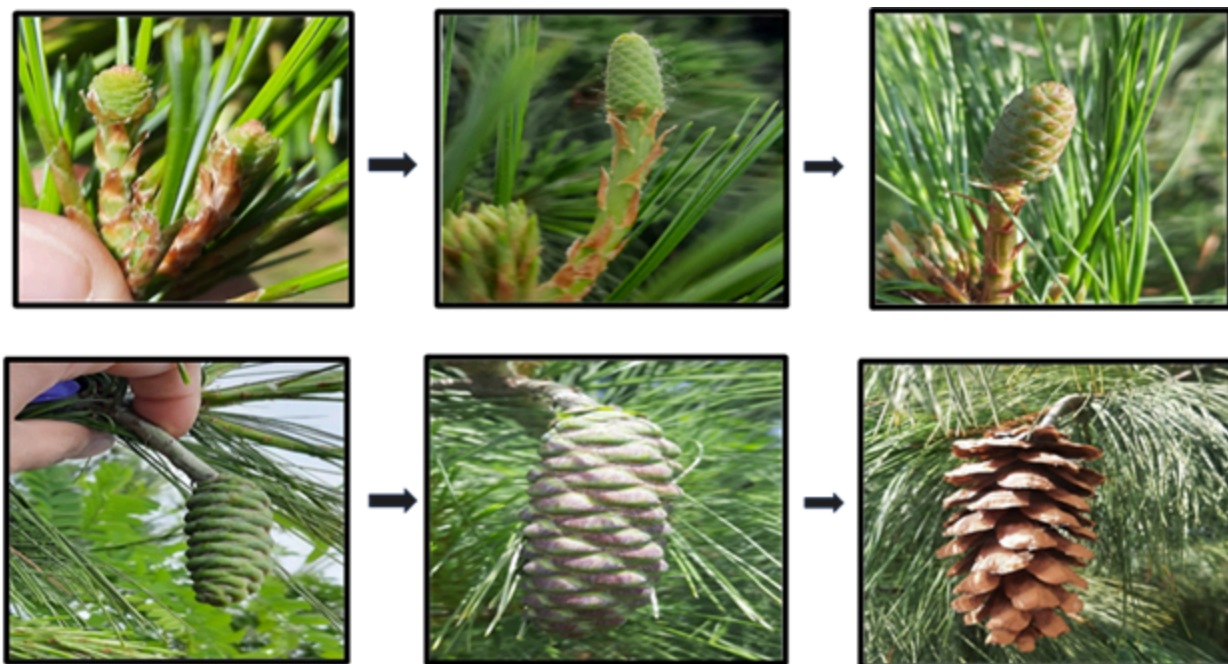


Figure 2

Reproductive phenophases of female strobili