

Effect of altitude and slope on the germination and growth of blue pine (*Pinus wallichiana* A. B. Jacks.) in reference to stratified and non-stratified seeds

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Abstract

During the present study, the impact of cold stratification at different elevations and angles on the germination and growth characteristics of white pine (*Pinus wallichiana* A. B. Jacks.) seedlings were examined. In the years 2019–20, seeds were gathered in Himachal Pradesh from several altitudinal ranges [1800–2100 (A₁), 2100–2400 (A₂), 2400–2700 (A₃), and > 2700 (A₄) m.a.s.l.] and aspects [southern aspect (As₁), eastern aspect (As₂), and south-eastern aspect (As₃)]. The nursery was established in 2020 at the Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (HP), Department of Silviculture and Agroforestry Forest Nursery. After being sun-dried, the cones were manually shaken and twisted to release the seeds. To break their dormancy, seeds were stratified with various layers of sand and moss for around 60 days before sowing. Seed viability (78.9% in S, 63.1 in NS), germination percentage (84.3% in S, 80.8 in NS), seedling height (11.7 in S, 9.22 cm in NS), survival percentage (71.3% in S, 59.6% in NS), and other growth traits of stratified (S) and non-stratified (NS) seeds were found to be significantly higher in stratified seeds collected from lower altitudinal ranges. Based on the findings, it was concluded that cold stratification of seeds obtained from lower altitude sites was suitable for extensive planting owing to their higher vitality and overall superior health.

Introduction

As is widely known, environmental conditions change in predictable ways over altitudinal gradients. Many plant life cycles involve a shift from seedling to adult that is fraught with danger (Lohengrin et al. 2000). Stress conditions imposed by temperature increases (maximum or minimum) and drought are likely to affect the important characteristics of tree populations acclimated to a particular altitude and aspect. In general, low-altitude zones are associated with higher temperatures and greater hydrologic stress, whereas higher-altitude zones can experience a larger frequency of frosts (Saenz-Romero et al. 2006; Rehfeldt 1988). These divergent climatic conditions can result in distinct genetic and phenotypic alterations within populations of certain conifer species, (Viveros-Viveros et al. 2005; Saenz-Romero et al. 2006; Viveros-Viveros et al. 2009). The mechanisms regulating the timing of this change would be subject to tremendous selective pressure. If seed germination is an adaptive process, natural selection ought to favor seed germination patterns that reduce the probability of encountering inappropriate environmental conditions during seedling establishment (Lohengrin et al. 2000). Under this scenario, how seeds germinate should depend on the environment and the likelihood of seedling mortality in that environment. The majority of research on the association between germination patterns and habitat characteristics has focused on comparisons between distinct species (Lohengrin et al. 2000). Examining how various populations of the same species in different habitats germinate their seeds is an effective method for achieving this goal. This strategy maintains the majority of the other aspects of a species life history, making it easier to identify changes in habitat (Venable 1984).

A well-known attribute of altitudinal gradients is the fall in ambient temperature with altitude. If germination patterns change in response to habitat conditions, it would be reasonable to expect seeds from higher elevations to be better adapted to germinate under low-temperature conditions. However,

numerous studies have revealed that the optimal germination temperatures for many tundra species are surprisingly high (20°C–30°C) compared to ambient temperatures (Lohengrin et al. 2000). Jull and Blaich (2000), researchers say that even under optimal environmental conditions, conifer seed exhibits great dormancy. Meyer and Mosen (1991) hypothesize that populations generally subjected to longer duration of snow cover and harsh winter conditions will require longer stratification times for germination than populations exposed to milder winters. They reasoned that such a function would enable seeds to detect the amount of time spent in winter conditions, allowing them to time germination to an optimal period for seedling survival and establishment.

Under natural conditions, the requirement for stratification prevents precocious germination in autumn, when optimal moisture and temperature regimes for seed germination and seedling establishment are unlikely to persist for more than a few days, allowing germination to occur only after seeds have been exposed to low winter temperatures. This also prevents freezing temperatures from damaging early seedlings (Baskin and Baskin 2004). In addition, cold stratification has long been regarded as a means of overcoming physiological dormancy in the seeds of numerous species. Natural regeneration in conifers is nearly dependent on seeds, whose production, dispersal, germination capacity, and seedling establishment success depend on seed production, dispersal, germination capacity, and establishment success. Seed viability testing is also essential for determining the quality of seeds, which has a significant impact on planting stock quality. If the seed's quality is poor at the time of sowing, no amount of post-sowing treatment will be effective. Before beginning the sowing and planting process, it is essential to have clarity regarding the seed's quality and vitality (Ajaz-ul-Islam et al. 2005).

The blue or Bhutan pine, *Pinus wallichiana* (A.B. Jacks) is an ecologically and economically significant conifer tree species of the Pinaceae family. It is widely referred to as Kail in the trade, is native to the Himalayas from Kashmir to Bhutan, and typically grows between 1500–3000 m asl and up to 3400 m asl in Bhutan (Farjon 2013). Like other broad-leaved plants, it generally prefers a southern exposure. Conifers include *Pinus wallichiana*, *Cedrus deodara*, *Taxus baccata*, *Picea smithiana*, and *Abies pindrow*, which coexist alongside broad-leaved species such as *Quercus*, *Rhododendron*, *Aesculus*, *Acer*, *Betula*, etc. (Bhattacharyya et al. 1998). A previous study done by Lopoez et al. (2017) focused only on the effect that various elevations had on the offspring of *Pinus wallichiana* concerning the climatic stress index. The present study has investigated the influence of altitude and aspect on *P. wallichiana* offspring after and before seed stratification treatment and correlated this with the stress condition index to check the impact of climate. Therefore, that study could be helpful to understand the seed stratification treatment at different elevations.

Materials And Methods

Explanation of the study

The experiment was carried out at the Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (HP), Department of Silviculture and Agroforestry Forest nursery in 2020. The nursery site is located 1200

m above mean sea level in the north-west Himalaya between 30° 51' N latitude and 76° 11' E longitude (Survey of Indian Toposheet Number 53F/1). The experimental region is hilly, marked by rises and depressions, and has a gradual south-eastward slope. The area experiences temperatures ranging from 1°C in the winter to 33°C during the warmest months of May and June. The yearly precipitation ranges from 0 to 342 mm, with the greatest precipitation during the monsoon season (July–September). To conduct the studies, *P. wallichiana* trees at various elevations ranging from 1800 to 2100 (m asl) to more than 2700 (m.a.s.l) were selected (Table 1). Ten trees of *P. wallichiana* were selected having diameter of 50 to 60 cm for sample collection and visual assessment in terms of health status.

Table 1
Geographic and climatic details of the selected seed sources of *P. wallichiana*

District	Place	Latitude	Longitude	Relative humidity (%)	Mean temperature (C)	Mean annual rainfall (Kg/m ²)
Kullu	Kotadhar	31.3928	77.4402	61.48	10.90	2054.6
Kullu	Naggar	32.114	77.1643	71.91	16.60	1953.5
Sirmaur	Churdhar	30.8763	77.4013	58.42	16.28	1681.6
Sirmaur	Devamanal	30.770	77.4329	57.52	17.39	1523.4
Sirmaur	Naura Forest Division	30.8139	77.4220	57.92	16.91	1593.8
Shimla	Chail	30.9697	77.1975	57.76	17.06	1476.3
Shimla	Kufri	31.0979	77.2078	58.54	15.97	1605.2
Shimla	Mashobra	31.3077	77.2259	58.80	15.55	1665.9
Shimla	Narkanda	31.2578	77.4602	59.66	11.30	2111.9

Methodology Adopted

Three slopes such as the southern, eastern, and south-eastern slopes were selected for sampling. The observations were recorded at four altitudinal sites: A₁; 1800–2100 m asl, A₂; 2100–2400 m asl, A₃; 2400–2700 m asl, and A₄; >2700 m asl Cones were collected randomly from phenotypically superior trees that were at least 30 m apart in (mid-October–mid-November). The seeds were extracted by shaking and manually twisting the cones after they were sun-dried. Before seeding, seeds were stratified with different layers of sand and moss for around 60 days to break their dormancy (Plate 1). After the stratification phase was complete, the seeds were directly sown in root trainers containing a mixture of deodar forest soil and farmyard manure (FYM) in a ratio of 2:1 and then placed under nursery conditions. The sown seeds were managed by applying the necessary management procedures, such as irrigation and weeding.

Observation Recorded

The seed viability was determined by using the Tetrazolium test (Tz) by taking 50 seeds in each replication. Seeds with fully stained (red) embryos and other tissues are deemed viable (Bonner 1974).

$$\text{Percentviability} = \frac{\text{Numberofviableseeds}}{\text{Totalnumberofseeds}} \times 100$$

The daily observations were documented by counting the number of germinated seeds. Counts of germination were determined using the 2x rule (radiate length reached two times the length of the seed). After the test period, the ungerminated seeds were moved to a laboratory, where the embryo's health was determined by severing the seeds and observing whether or not the embryo appeared healthy. The percentage of germination, germination capacity, germination energy, germination rate, and germination value were estimated (as per the procedure prescribed by the International Feel Testing Association, ISTA, 1985). A peak value was determined as the maximum mean daily germination attained during the test period (Czabator 1962). The mean germination time was computed according to Djavanshir and Pourbeik (1976).

To evaluate progeny attributes, 10 random seedlings from each replication were carefully uprooted without breaking the roots, twelve months after the seeds were sown. Seedling heights (cm) and root lengths (cm) were measured with a meter scale in cm, seedling diameter (mm) was measured with a digital vernier calliper, needle area (cm²) was determined with a CI-202 Leaf Area Meter, shoot/root biomass (the biomass content was determined on a dry weight basis following ISTA rule 1999), and total seedling dry weight was calculated by adding root and shoot dry weight in g. The seedling vigour index was obtained by multiplying the total length of the seedling (length of hypocotyls plus length of radical) by the total germination percent (Bhattacharya et al. 1991). The survival percentage was calculated by using the formula (Pattanaik et al. 2015):

$$\text{Survival (\%)} = \frac{\text{Total percent germination}}{\text{Number of seeds germinated in that seed source}} \times 100$$

To ascertain an individual's health status, a stress condition index (SCI) was established using visual indicators such as foliage, branches, and crown characteristics (SCI = F + B + C). Where F denotes the percentage of dead foliage relative to total standing foliage, B denotes the percentage of branches without foliage that appears to be supposed to have foliage, and C denotes the percentage of the crown that is no longer alive, measured from the first branch with live needles to the top. Each trait has a value between zero and five. Zero indicates that the features are in outstanding condition (all five are alive); while five indicates that they are in a dreadful form. Thus, the SCI ranges from 0 to 15, with 0 indicating optimum health and 15 indicating the highest level of stress (Lopez et al. 2017).

Statistical analysis

The experimental data were analyzed using the analysis of variance approach for a two-factor randomized block design, as outlined by Gomez and Gomez (1984), with three replications for each *Pinus wallichiana* seed source. The estimated "F" values were compared to the tabulated "F" values at a 5% significance level. The difference is considered significant if the calculated value exceeds the tabulated value. The Duncan test ($P \leq 0.05$) was conducted to determine whether there was a statistically significant difference between the mean values of the various treatments. To measure the strength of the linear relationship among the parameters as dependent variables, correlation coefficients (Karl Pearson's) will also be assessed. All the traits were evaluated and recorded using statistical software (SPSS 20.0, Microsoft Excel, and R-studio).

Results

Germination parameters

Seed germination is influenced by the prevailing ecological circumstances in the habitat; it is dependent on a variety of environmental parameters, such as light, temperature, and moisture content of the germination medium. It is generally known that seeds of different varieties and origins of the same species have varying temperature responses, and it is possible to assume that these responses are adaptive. The success or failure of a population in a specific environment is reliant on how well its germination responses match the ecological requirements of its habitat. To determine the effect of climatic circumstances, daily germination was documented in this study. After sowing seeds in February 2020, daily germination was examined, with maximum germination occurring between (50–75 days) for stratified seeds (Fig. 1) and (75–95 days) for non-stratified seeds (Fig. 2). Up to twenty days, daily mean germination was observed. Based on daily counts, A₁ altitude (1800–2100 m asl) was reported to have the highest seed germination, followed by A₂ altitude (2100–2400 m asl). The daily germination rate is highest for seeds taken from a southern aspect, followed by a southeastern aspect. Peak germination occurred between 9 and 11 days for S seeds and 7 days for NS seeds during the test periods shown in Fig. 3.

P. wallichiana germination parameters are affected by differences in altitude, aspect, and stratification (Fig. 4). In A₂ altitude, S and NS seeds exhibited the highest seed viability (78.7% and 63.3%, respectively), and different altitudes conformed to the following trends: A₂ > A₁ > A₄ ≥ A₃. The seeds facing south and south-southeast had the maximum viability. The A₁ altitudinal range has the highest germination capacity for S and NS seeds (90.6% and 83.5%, respectively) when compared to other altitudes. Each of the elevational ranges was distinguishable from the others. The S seeds have the highest germination capacity from the southern aspect (83.7%) and the NS seeds from the southeastern aspect (77.1%). Similarly, the germination percentage of S and NS seeds follows the same trends as germination capacity. Significantly more germination energy was present in the S and NS seeds of origin A₂ (35.7 and 32.2%, respectively) than in those of origins A₁, A₃, and A₄ altitudes. A₂ and A₁ altitudes yielded the highest germination values (14.9 and 10.9) for *Pinus wallichiana* S and NS seeds. The southern and

southeastern aspects exhibited the highest germination value among the other aspects. In terms of the average effect, the A₁ gradient had the quickest germination speed (3.42), followed by A₂, A₃, and A₄.

Growth Parameters

The growth parameters of stratified seeds of *P. wallichiana* were affected by differences in altitude, aspect, and cold stratification (Plate 2; Table 2). The progenies of the A₁ altitudinal range produced the tallest seedlings (11.7 cm), which were significantly higher than those from the A₂, A₃, and A₄ altitudinal ranges. The range of elevation between A₂ and A₃ remained statistically identical. Similar to seedling height, only the effect of the altitude gradient had a significant effect on collar diameter. Maximum collar diameter growth (2.68 mm) was seen in the seedling of the A₂ altitudinal gradient, which statistically remained identical to only A₁ and A₃. The highest needle area (37.6 cm²) was reported in the A₁ altitudinal range, which was statistically identical to the A₂ range (36.3 cm²). The southern aspect (As₁) had the greatest needle surface area (38.7 cm²) among the four aspects, followed by the south-eastern (34.3 cm²) and eastern aspects (29.8 cm²), respectively. Maximum needle surface area was recorded in the treatment combination A₁As₁ (45.7 cm²/needle), which was comparable to A₂As₁. Root length was reported to be higher in A₁ (21.2 cm) altitudinal range than all other altitudinal ranges, while both A₃ and A₄ remained statistically equivalent. The treatment combination A₁As₁, which was identical to A₁As₂, yielded the greatest root length (25.6 cm). The highest shoot-to-root ratio was found at altitude A₁ (2.36), which was statistically equivalent to A₃ (2.10) compared to other altitude ranges following the trends A₁ > A₃ > A₂ > A₄. A₁ altitude seedlings accumulated the greatest amount of dry seedling weight (0.47 g), which was significantly more than all other altitude gradients. Seedlings from the A₁ altitudinal range exhibited the highest survival percentage (71.3%), followed by those from A₂, A₃, and A₄ in descending order. The A₃ and A₄ altitudinal gradients retained statistically identical values. The highest survival percentage was observed with treatment combination A₁As₂ (78.2%), while the lowest value was observed with A₄As₂ (28.9%), which was comparable to A₃As₂ (30.9%). The seedling vigour index follows the following trends: A₁ (1796) > A₂ (1286) > A₃ (880) > A₄ (689). Among aspects, the southern aspect (As₁) demonstrated the highest value of the seedling vigour index (1101), followed by the south-eastern (As₃) and eastern (As₂) sides, respectively. Treatment combination A₁As₁ (2298) had the highest value for Stress Vigour Index.

Table 2

Effect of altitudes, aspects and their interaction on growth parameters of stratified seeds of *P. wallichiana* tree

Growth parameters	Altitude	Aspects (Mean $\pm$ Std. Deviation)			
		As ₁	As ₂	As ₃	Mean
Seedling height (cm)	A ₁	12.28 $\pm$ 1.91	12.1 $\pm$ 0.96	10.9 $\pm$ 0.54	11.7 ^C $\pm$ 1.27
	A ₂	9.66 $\pm$ 1.45	10.1 $\pm$ 1.84	9.43 $\pm$ 0.98	9.72 ^B $\pm$ 1.30
	A ₃	8.66 $\pm$ 1.44	9.56 $\pm$ 0.08	9.76 $\pm$ 0.77	9.33 ^B $\pm$ 0.96
	A ₄	8.06 $\pm$ 1.80	7.62 $\pm$ 0.73	7.86 $\pm$ 1.61	7.85 ^A $\pm$ 1.28
	Mean	9.66 $\pm$ 2.20	9.83 $\pm$ 1.91	9.49 $\pm$ 1.45	
P value _(0.05)		Alt = 0.00 (S); Asp = 0.65 (NS); Alt $\times$ Asp = 0.36 (NS)			
Collar diameter (mm)	A ₁	2.67 $\pm$ 0.25	2.69 $\pm$ 0.15	2.54 $\pm$ 0.20	2.63 ^B $\pm$ 0.19
	A ₂	2.65 $\pm$ 0.33	2.63 $\pm$ 0.23	2.76 $\pm$ 0.27	2.68 ^B $\pm$ 0.25
	A ₃	2.72 $\pm$ 0.33	2.41 $\pm$ 0.26	2.70 $\pm$ 0.36	2.61 ^B $\pm$ 0.32
	A ₄	2.58 $\pm$ 0.16	2.19 $\pm$ 0.11	2.25 $\pm$ 0.06	2.34 ^A $\pm$ 0.21
	Mean	2.66 $\pm$ 0.24	2.48 $\pm$ 0.27	2.56 $\pm$ 0.30	
P value _(0.05)		Alt = 0.02 (S); Asp = 0.20 (NS); Alt $\times$ Asp = 0.45 (NS)			
Needle area (cm ²)	A ₁	45.7 ^{Cc} $\pm$ 1.25 (c)	30.4 ^{Aa} $\pm$ 0.10 (ab)	36.7 ^{Cb} $\pm$ 1.35 (b)	37.6 ^B $\pm$ 6.74
	A ₂	43.8 ^{BCb} $\pm$ 7.10 (c)	31.5 ^{Aa} $\pm$ 1.25 (ab)	33.6 ^{ABa} $\pm$ 1.15 (ab)	36.3 ^B $\pm$ 6.77
	A ₃	29.9 ^{Aa} $\pm$ 6.55 (ab)	27.5 ^{Aa} $\pm$ 4.05 (a)	35.3 ^{BCa} $\pm$ 0.75 (b)	30.9 ^A $\pm$ 5.16

** Uppercase of alphabets in superscript used for altitude and lowercase for aspects, alphabets in parenthesis are used for overall interaction effects. Value with same alphabet means statistically similar

Growth parameters	Altitude	Aspects (Mean $\pm$ Std. Deviation)			
		As ₁	As ₂	As ₃	Mean
	A ₄	35.5 ^{ABa} $\pm$ 3.05 (b)	29.9 ^{Aa} $\pm$ 5.05 (ab)	31.5 ^{Aa} $\pm$ 1.75 (ab)	32.3 ^A $\pm$ 3.93
	Mean	38.74 ^c $\pm$ 7.96	29.8 ^a $\pm$ 3.18	34.3 ^b $\pm$ 2.30	
P value _(0.05)		Alt = 0.00 (S); Asp = 0.00 (S); Alt $\times$ Asp = 0.00 (S)			
Root length (cm)	A ₁	25.6 ^{Cb} $\pm$ 2.80 (d)	21.1 ^{Cab} $\pm$ 0.95 (cd)	16.8 ^{Aa} $\pm$ 6.13 (bc)	21.2 ^C $\pm$ 5.13
	A ₂	18.6 ^{BCa} $\pm$ 5.97 (bc)	16.8 ^{Ba} $\pm$ 1.77 (bc)	14.9 ^{Aa} $\pm$ 5.22 (abc)	16.8 ^B $\pm$ 4.37
	A ₃	12.4 ^{ABa} $\pm$ 3.02 (ab)	14.9 ^{Ba} $\pm$ 0.81 (abc)	13.2 ^{Aa} $\pm$ 2.61 (ab)	13.5 ^A $\pm$ 2.30
	A ₄	8.69 ^{Aa} $\pm$ 3.21 (a)	9.32 ^{Aa} $\pm$ 1.22 (a)	15.5 ^{Aa} $\pm$ 4.95 (abc)	11.2 ^A $\pm$ 4.42
	Mean	16.3 $\pm$ 7.52	15.5 $\pm$ 4.54	15.1 $\pm$ 4.39	
P value _(0.05)		Alt = 0.00 (S); Asp = 0.55 (NS); Alt $\times$ Asp = 0.00 (S)			
Number of primary roots	A ₁	7.0 ^{Ba} $\pm$ 1.0 (cd)	8.33 ^{Cab} $\pm$ 0.57 (d)	6.67 ^{Ba} $\pm$ 0.57(cd)	7.33 ^C $\pm$ 0.88
	A ₂	4.67 ^{Aa} $\pm$ 1.52 (ab)	6.0 ^{Bb} $\pm$ 1.0 (bc)	7.67 ^{Bb} $\pm$ 0.57 (cd)	6.11 ^{BC} $\pm$ 1.50
	A ₃	6.0 ^{Bb} $\pm$ 1.0(bc)	5.33 ^{Bab} $\pm$ 1.52 (b)	4.33 ^{Aa} $\pm$ 0.57 (ab)	5.22 ^B $\pm$ 0.83
	A ₄	3.33 ^{Aa} $\pm$ 0.57 (a)	3.0 ^{Aa} $\pm$ 1.73 (a)	3.33 ^{Aa} $\pm$ 1.15 (a)	3.22 ^A $\pm$ 0.19
	Mean	5.24 $\pm$ 1.60	5.66 $\pm$ 2.18	5.5 $\pm$ 2.01	
P value _(0.05)		Alt = < 0.01 (S); Asp = 0.65 (NS); Alt $\times$ Asp = 0.02 (S)			
Number of secondary roots	A ₁	7.67 $\pm$ 1.53	9.67 $\pm$ 0.58	11.3 $\pm$ 2.08	9.56 ^C $\pm$ 2.07
	A ₂	7.00 $\pm$ 2.00	7.33 $\pm$ 1.15	7.00 $\pm$ 1.00	7.11 ^B $\pm$ 1.27
** Uppercase of alphabets in superscript used for altitude and lowercase for aspects, alphabets in parenthesis are used for overall interaction effects. Value with same alphabet means statistically similar					

Growth parameters	Altitude	Aspects (Mean $\pm$ Std. Deviation)			
		As ₁	As ₂	As ₃	Mean
	A ₃	8.67 $\pm$ 1.15	8.67 $\pm$ 0.58	9.67 $\pm$ 6.03	9.00 ^{BC} $\pm$ 3.12
	A ₄	5.00 $\pm$ 2.00	4.00 $\pm$ 1.00	4.33 $\pm$ 1.53	4.44 ^A $\pm$ 1.42
	Mean	7.08 $\pm$ 2.02	7.42 $\pm$ 2.35	8.08 $\pm$ 3.96	
P value _(0.05)		Alt = 0.00 (S); Asp = 0.51 (NS); Alt $\times$ Asp = 0.67 (NS)			
Number of tertiary roots	A ₁	15.0 ^{Ca} $\pm$ 3.0 (f)	12.0 ^{Ba} $\pm$ 2.0 (e)	11.7 ^{Ba} $\pm$ 0.57 (e)	12.9 ^C $\pm$ 1.83
	A ₂	8.67 ^{Ba} $\pm$ 1.15 (cd)	10.3 ^{Bb} $\pm$ 0.57 (cd)	11.3 ^{Bb} $\pm$ 1.15 (e)	10.1 ^B $\pm$ 1.35
	A ₃	4.3 ^{Ab} $\pm$ 0.57 (b)	0 (a)	0 (a)	1.44 ^A $\pm$ 2.5
	A ₄	0 (a)	0 (a)	0 (a)	0
	Mean	7.0 ^a $\pm$ 6.4	5.58 ^{ab} $\pm$ 6.48	5.75 ^b $\pm$ 6.64	
P value _(0.05)		Alt = 0.00 (S); Asp = 0.01 (S); Alt $\times$ Asp = 0.00 (S)			
Fresh root weight (gm)	A ₁	0.28 $\pm$ 0.04	0.25 $\pm$ 0.07	0.36 $\pm$ 0.06	0.36 ^C $\pm$ 0.06
	A ₂	0.25 $\pm$ 0.06	0.20 $\pm$ 0.03	0.26 $\pm$ 0.05	0.26 ^B $\pm$ 0.05
	A ₃	0.14 $\pm$ 0.02	0.12 $\pm$ 0.03	0.16 $\pm$ 0.02	0.16 ^A $\pm$ 0.02
	A ₄	0.15 $\pm$ 0.01	0.12 $\pm$ 0.01	0.15 $\pm$ 0.03	0.15 ^A $\pm$ 0.03
	Mean	0.21 ^b $\pm$ 0.07	0.17 ^a $\pm$ 0.07	0.23 ^b $\pm$ 0.10	
P value _(0.05)		Alt = 0.00 (S); Asp = 0.02 (S); Alt $\times$ Asp = 0.37 (NS)			
Dry root weight (gm)	A ₁	0.12 $\pm$ 0.02	0.11 $\pm$ 0.03	0.14 $\pm$ 0.01	0.23 ^B $\pm$ 0.10

** Uppercase of alphabets in superscript used for altitude and lowercase for aspects, alphabets in parenthesis are used for overall interaction effects. Value with same alphabet means statistically similar

Growth parameters	Altitude	Aspects (Mean $\pm$ Std. Deviation)			
		As ₁	As ₂	As ₃	Mean
	A ₂	0.07 $\pm$ 0.03	0.09 $\pm$ 0.03	0.09 $\pm$ 0.03	0.14 ^A $\pm$ 0.01
	A ₃	0.08 $\pm$ 0.02	0.07 $\pm$ 0.01	0.10 $\pm$ 0.01	0.09 ^A $\pm$ 0.03
	A ₄	0.09 $\pm$ 0.02	0.14 $\pm$ 0.03	0.09 $\pm$ 0.03	0.10 ^A $\pm$ 0.01
	Mean	0.09 $\pm$ 0.03	0.09 $\pm$ 0.02	0.10 $\pm$ 0.03	
P value _(0.05)		Alt = 0.00 (S); Asp = 0.10 (NS); Alt $\times$ Asp = 0.84 (NS)			
Fresh shoot weight (gm)	A ₁	0.45 $\pm$ 0.06	0.39 $\pm$ 0.06	0.59 $\pm$ 0.02	0.48 ^C $\pm$ 0.10
	A ₂	0.35 $\pm$ 0.05	0.43 $\pm$ 0.07	0.49 $\pm$ 0.23	0.42 ^{BC} $\pm$ 0.14
	A ₃	0.38 $\pm$ 0.07	0.34 $\pm$ 0.06	0.38 $\pm$ 0.03	0.37 ^A $\pm$ 0.05
	A ₄	0.27 $\pm$ 0.07	0.25 $\pm$ 0.04	0.36 $\pm$ 0.08	0.30 ^A $\pm$ 0.08
	Mean	0.36 ^a $\pm$ 0.09	0.35 ^a $\pm$ 0.09	0.46 ^b $\pm$ 0.14	
P value _(0.05)		Alt = 0.00 (S); Asp = 0.01 (S); Alt $\times$ Asp = 0.41 (NS)			
Dry shoot weight (gm)	A ₁	0.22 ^{Aa} $\pm$ 0.03 (ab)	0.28 ^{Aab} $\pm$ 0.03 (bc)	0.33 ^{Bb} $\pm$ 0.05 (c)	0.33 ^C $\pm$ 0.05
	A ₂	0.21 ^{Aa} $\pm$ 0.05 (ab)	0.22 ^{ABa} $\pm$ 0.09 (ab)	0.18 ^{Aa} $\pm$ 0.04 (ab)	0.18 ^{AB} $\pm$ 0.04
	A ₃	0.22 ^{Aa} $\pm$ 0.03 (ab)	0.16 ^{Aa} $\pm$ 0.03 (a)	0.20 ^{Aa} $\pm$ 0.05 (a)	0.20 ^B $\pm$ 0.05
	A ₄	0.17 ^{Aa} $\pm$ 0.06 (a)	0.14 ^{Aa} $\pm$ 0.03 (a)	0.17 ^{Aa} $\pm$ 0.03 (a)	0.17 ^A $\pm$ 0.03
	Mean	0.21 $\pm$ 0.04	0.20 $\pm$ 0.07	0.22 $\pm$ 0.08	
P value _(0.05)		Alt = 0.00 (S); Asp = 0.62 (NS); Alt $\times$ Asp = 0.06 (S)			

** Uppercase of alphabets in superscript used for altitude and lowercase for aspects, alphabets in parenthesis are used for overall interaction effects. Value with same alphabet means statistically similar

Growth parameters	Altitude	Aspects (Mean $\pm$ Std. Deviation)			
		As ₁	As ₂	As ₃	Mean
Shoot/Root biomass Ratio	A ₁	1.84 $\pm$ 0.47	2.60 $\pm$ 0.66	2.36 $\pm$ 0.49	2.36 ^{AB} $\pm$ 0.49
	A ₂	2.99 $\pm$ 0.37	2.65 $\pm$ 0.91	2.00 $\pm$ 0.25	2.00 ^A $\pm$ 0.25
	A ₃	2.97 $\pm$ 0.79	2.18 $\pm$ 0.06	2.10 $\pm$ 0.50	2.10 ^{AB} $\pm$ 0.50
	A ₄	1.88 $\pm$ 0.39	1.95 $\pm$ 0.22	1.98 $\pm$ 0.31	1.98 ^A $\pm$ 0.31
	Mean	2.42 $\pm$ 0.74	2.34 $\pm$ 0.58	2.11 $\pm$ 0.38	
P value _(0.05)		Alt = 0.11 (S); Asp = 0.34 (NS); Alt $\times$ Asp = 0.41 (NS)			
Total seedling dry weight (gm)	A ₁	0.34 $\pm$ 0.02	0.40 $\pm$ 0.03	0.47 $\pm$ 0.04	0.47 ^B $\pm$ 0.04
	A ₂	0.29 $\pm$ 0.08	0.31 $\pm$ 1.10	0.27 $\pm$ 0.06	0.27 ^A $\pm$ 0.06
	A ₃	0.30 $\pm$ 0.03	0.23 $\pm$ 0.04	0.30 $\pm$ 0.05	0.30 ^A $\pm$ 0.05
	A ₄	0.25 $\pm$ 0.07	0.21 $\pm$ 0.05	0.25 $\pm$ 0.05	0.25 ^A $\pm$ 0.05
	Mean	0.30 $\pm$ 0.06	0.29 $\pm$ 0.09	0.32 $\pm$ 0.10	
P value _(0.05)		Alt = 0.00 (S); Asp = 0.27 (NS); Alt $\times$ Asp = 0.134 (NS)			
Survival (%)	A ₁	69.3 ^{Ba} $\pm$ 8.11 (de)	78.2 ^{Ba} $\pm$ 7.81 (e)	66.2 ^{Ba} $\pm$ 3.08 (cde)	71.3 ^C $\pm$ 7.95
	A ₂	60.4 ^{ABa} $\pm$ 7.34 (bcde)	61.3 ^{Ba} $\pm$ 10.9 (bcde)	56.0 ^{ABa} $\pm$ 2.31 (bcd)	59.3 ^B $\pm$ 7.12
	A ₃	51.1 ^{Aa} $\pm$ 9.65 (bcd)	30.7 ^{Aa} $\pm$ 13.9 (a)	51.6 ^{ABa} $\pm$ 10.1 (bcd)	44.4 ^A $\pm$ 14.3
	A ₄	49.3 ^{Aa} $\pm$ 3.53 (bc)	28.9 ^{Aa} $\pm$ 18.9 (a)	44.9 ^{Aa} $\pm$ 11.1 (ab)	41.1 ^A $\pm$ 14.5
	Mean	57.6 $\pm$ 10.5	49.8 $\pm$ 24.6	54.7 $\pm$ 10.5	

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Growth parameters	Altitude	Aspects (Mean ± Std. Deviation)			
		As ₁	As ₂	As ₃	Mean
P value _(0.05)		Alt = 0.00 (S); Asp = 0.13 (NS); Alt × Asp = 0.03 (S)			
Seedling vigor index	A ₁	2298 ^{Bb} ± 269 (e)	1719 ^{Cab} ± 73.4 (d)	1370 ^{Aa} ± 495.69 (cd)	1796 ^C ± 496
	A ₂	1672 ^{Ba} ± 542 (d)	1025 ^{Ba} ± 185 (abc)	1162 ^{Aa} ± 436.11 (bcd)	1286 ^B ± 466
	A ₃	901 ^{Aa} ± 238 (abc)	853 ^{Ba} ± 91.5 (abc)	886 ^{Aa} ± 293.21 (abc)	880 ^A ± 196
	A ₄	569 ^{Aa} ± 252 (ab)	509 ^{Aa} ± 111 (a)	988 ^{Aa} ± 337.12 (abc)	689 ^A ± 314
	Mean	1360 ^b ± 764	1026 ^a ± 472	1101.6 ^a ± 390.4	
P value _(0.05)		Alt = 0.00 (S); Asp = 0.01 (S); Alt × Asp = 0.00(S)			
** Uppercase of alphabets in superscript used for altitude and lowercase for aspects, alphabets in parenthesis are used for overall interaction effects. Value with same alphabet means statistically similar					

The growth parameters of non-stratified seeds of *P. wallichiana* were affected by differences in altitude and aspect as well as by cold stratification (Plate 3; Table 3). The progenies of the A₂ altitudinal range produced the tallest seedlings (9.22 cm), which were shown to be statistically equivalent to the A₁ range. Maximum collar diameter growth was seen in the seedling of the A₂ altitudinal gradient (2.54 mm), which statistically stayed the same as A₁. The collar diameter of seedlings at various elevations grew as follows: A₂ > A₁ > A₃ > A₄. Regardless of the aspect, the A₁ altitudinal range had the greatest needle area (34.06 cm²), which was statistically identical to the A₂. The treatment combination A₁As₁ demonstrated the greatest needle surface area (39.65 cm²). Maximum root length (12.92 cm) was measured in the A₂ altitudinal range, which differed significantly from all other altitudinal ranges, while the southeastern aspect displayed the greatest root length (12.47 cm). The shoot-root ratio was recorded as highest in A₂ (2.51) altitude. Maximum seedling dry weight was acquired by A₁ (0.23 g), which was found to be considered equivalent to A₂ (0.20 g); however, the southeastern aspect exhibits the highest value. The seedlings of the A₁ altitudinal range demonstrated the highest survival rate (59.6%), followed by those of the A₂, A₃, and A₄ ranges in declining order. The SVI at various altitudes followed this pattern: A₂ (1006), A₁ (973), A₃, and A₄ (500). The southeastern aspect (As₃) demonstrated the highest value of the seedling vigour index (928), followed by the southern (As₁) and eastern (As₂) perspectives, respectively.

Table 3
Effect of altitudes, aspects and their interaction on growth parameters of non-stratified seeds of *P. wallichiana* tree

Growth parameters	Altitude	Aspects (Mean $\pm$ Std. Deviation)			
		As ₁	As ₂	As ₃	Mean
Seedling height (cm)	A ₁	9.18 $\pm$ 0.37	7.61 $\pm$ 2.53	9.52 $\pm$ 0.23	8.77 ^B $\pm$ 1.56
	A ₂	9.23 $\pm$ 0.75	9.08 $\pm$ 0.60	9.35 $\pm$ 0.51	9.22 ^B $\pm$ 0.56
	A ₃	8.05 $\pm$ 1.39	7.36 $\pm$ 0.72	7.43 $\pm$ 1.05	7.62 ^A $\pm$ 1.00
	A ₄	7.06 $\pm$ 0.94	5.77 $\pm$ 0.36	6.50 $\pm$ 1.40	6.44 ^B $\pm$ 1.03
	Mean	8.38 $\pm$ 1.23	7.46 $\pm$ 1.69	8.20 $\pm$ 1.55	
P value _(0.05)		Alt = 0.00 (S), Asp = 0.12(NS), Alt $\times$ Asp = 0.78 (NS)			
Collar diameter (mm)	A ₁	2.290 $\pm$ 0.14	2.47 $\pm$ 0.07	2.17 $\pm$ 0.22	2.310 ^{AB} $\pm$ 0.14
	A ₂	2.47 $\pm$ 0.43	2.36 $\pm$ 0.21	2.75 $\pm$ 0.20	2.524 ^B $\pm$ 0.20
	A ₃	2.367 $\pm$ 0.05	2.12 $\pm$ 0.27	2.23 $\pm$ 0.16	2.239 ^A $\pm$ 0.12
	A ₄	2.04 $\pm$ 0.16	2.247 $\pm$ 0.36	2.10 $\pm$ 0.32	2.130 ^A $\pm$ 0.10
	Mean	2.29 $\pm$ 0.18	2.298 $\pm$ 0.14	2.31 $\pm$ 0.29	
P value _(0.05)		Alt = 0.02 (S), Asp = 0.97 (NS), Alt $\times$ Asp = 0.24 (NS)			
Needle area (cm ²)	A ₁	39.65 ^{Ca} $\pm$ 7.15 (f)	30.57 ^{Aa} $\pm$ 10.47 (d)	31.95 ^{Aa} $\pm$ 1.35 (d)	34.06 ^B $\pm$ 7.66
	A ₂	32.55 ^{BCa} $\pm$ 0.95 (e)	33.15 ^{Aa} $\pm$ 5.35(e)	30.63 ^{Aa} $\pm$ 1.73 (d)	32.11 ^B $\pm$ 3.07
	A ₃	24.90 ^{Aa} $\pm$ 0.30 (a)	26.80 ^{Aa} $\pm$ 2.20 (b)	30.90 ^{Ab} $\pm$ 0.10 (d)	27.53 ^A $\pm$ 2.88
	A ₄	28.15 ^{ABa} $\pm$ 2.45 (bc)	23.75 ^{Aa} $\pm$ 2.45 (a)	27.91 ^{Aa} $\pm$ 5.13 (b)	26.60 ^A $\pm$ 3.76
	Mean	31.31 ^a $\pm$ 6.62	28.57 ^a $\pm$ 6.41	30.35 ^a $\pm$ 2.84	

** Uppercase of alphabets in superscript used for altitude and lowercase for aspects, alphabets in parenthesis are used for overall interaction effects. Value with same alphabet means statistically similar

Growth parameters	Altitude	Aspects (Mean ± Std. Deviation)			
		As ₁	As ₂	As ₃	Mean
P value _(0.05)		Alt = 0.00 (S), Asp = 0.25 (NS), Alt × Asp = 0.09 (S)			
Root length (cm)	A ₁	11.0 ± 1.44	11.4 ± 1.50	13.7 ± 2.52	12.04 ^{BC} ± 2.05
	A ₂	13.1 ± 1.79	11.7 ± 0.58	13.9 ± 1.94	12.92 ^C ± 1.68
	A ₃	11.5 ± 0.87	9.83 ± 0.29	12.1 ± 1.11	11.14 ^{AB} ± 1.24
	A ₄	10.4 ± 0.90	9.85 ± 0.72	10.2 ± 0.75	10.13 ^A ± 0.72
	Mean	11.50 ^{ab} ± 1.55	10.70 ^a ± 1.18	12.47 ^b ± 2.15	
P value _(0.05)		Alt = 0.00 (S), Asp = 0.02(S), Alt × Asp = 0.53 (NS)			
Number of primary roots	A ₁	4.33 ^{Ab} ± 0.58 (c)	5.67 ^{Aa} ± 0.58 (d)	5.33 ^{Aa} ± 0.58 (d)	5.11 ^A ± 0.69
	A ₂	4.33 ^{Ab} ± 0.58 (c)	3.00 ^{Bc} ± 1.0 (b)	5.33 ^{Aa} ± 0.58 (d)	4.22 ^B ± 1.17
	A ₃	3.67 ^{Ba} ± 2.08 (c)	3.00 ^{Ba} ± 1.0 (b)	1.67 ^{Cb} ± 0.58 (ab)	2.78 ^C ± 1.02
	A ₄	2.67 ^{BCa} ± 0.58 (b)	1.33 ^{Cb} ± 0.58 (a)	2.67 ^{Ba} ± 1.15 (b)	2.22 ^C ± 0.77
	Mean	3.75 ± 0.68	3.25 ± 1.55	3.75 ± 1.62	
P value _(0.05)		Alt = 0.00 (S), Asp = 0.23(NS), Alt × Asp = 0.002(S)			
Number of secondary roots	A ₁	6.67 ± 1.53	6.00 ± 2.0	7.00 ± 1.0	6.56 ^A ± 0.51
	A ₂	4.33 ± 1.53	1.67 ± 0.58	4.33 ± 0.58	3.44 ^B ± 1.54
	A ₃	2.67 ± 1.15	1.67 ± 0.58	1.33 ± 0.58	1.89 ^C ± 0.69
	A ₄	0.67 ± 0.58	0.00 ± 0.0	1.00 ± 1.0	0.56 ^D ± 0.51

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Growth parameters	Altitude	Aspects (Mean $\pm$ Std. Deviation)			
		As ₁	As ₂	As ₃	Mean
	Mean	3.58 ^a $\pm$ 2.20	2.33 ^{ab} $\pm$ 2.22	3.42 ^a $\pm$ 2.44	
P value _(0.05)		Alt = 0.00(S), Asp = 0.01(S), Alt $\times$ Asp = 0.25(NS)			
Number of tertiary roots	A ₁	4.00 ^{Ab} $\pm$ 2.0 (d)	4.33 ^{Ab} $\pm$ 0.58 (c)	5.00 ^{Ba} $\pm$ 0.58 (d)	4.44 ^A $\pm$ 0.51
	A ₂	4.33 ^{Ab} $\pm$ 0.58 (c)	2.33 ^{Bc} $\pm$ 0.58 (b)	6.33 ^{Aa} $\pm$ 0.58 (e)	4.33 ^A $\pm$ 2.0
	A ₃	2.33 ^{Ba} $\pm$ 0.58 (b)	0.00 ^{Cb} $\pm$ 0.0 (a)	0.00 ^{Cb} $\pm$ 0.0 (a)	0.78 ^B $\pm$ 1.35
	A ₄	0.00 ^{Ca} $\pm$ 0.0 (a)	0.00 ^{Ca} $\pm$ 0.0 (a)	0.00 ^{Ca} $\pm$ 0.0 (a)	0.00 ^C $\pm$ 0.0
	Mean	2.67 ^a $\pm$ 1.98	1.67 ^b $\pm$ 2.09	2.83 ^a $\pm$ 3.32	
P value _(0.05)		Alt = 0.00(S), Asp = 0.00(S), Alt $\times$ Asp = 0.00(S)			
Fresh root weight (gm)	A ₁	0.12 $\pm$ 0.05	0.15 $\pm$ 0.02	0.15 $\pm$ 0.02	0.14 ^B $\pm$ 0.03
	A ₂	0.11 $\pm$ 0.02	0.13 $\pm$ 0.03	0.15 $\pm$ 0.01	0.13 ^B $\pm$ 0.02
	A ₃	0.09 $\pm$ 0.01	0.09 $\pm$ 0.01	0.11 $\pm$ 0.03	0.10 ^A $\pm$ 0.02
	A ₄	0.08 $\pm$ 0.03	0.09 $\pm$ 0.04	0.07 $\pm$ 0.02	0.08 ^A $\pm$ 0.03
	Mean	0.10 $\pm$ 0.03	0.12 $\pm$ 0.04	0.12 $\pm$ 0.04	
P value _(0.05)		Alt = 0.00(S), Asp = 0.21(NS), Alt $\times$ Asp = 0.57 (NS)			
Dry root weight (gm)	A ₁	0.08 $\pm$ 0.02	0.08 $\pm$ 0.02	0.08 $\pm$ 0.02	0.08 ^A $\pm$ 0.02
	A ₂	0.08 $\pm$ 0.01	0.05 $\pm$ 0.04	0.07 $\pm$ 0.02	0.07 ^A $\pm$ 0.03
	A ₃	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.06 $\pm$ 0.01	0.05 ^B $\pm$ 0.01
	A ₄	0.06 $\pm$ 0.02	0.05 $\pm$ 0.04	0.06 $\pm$ 0.01	0.05 ^B $\pm$ 0.02
	Mean	0.06 $\pm$ 0.02	0.06 $\pm$ 0.03	0.06 $\pm$ 0.02	
P value _(0.05)		Alt = 0.01(S), Asp = 0.68(NS), Alt $\times$ Asp = 0.38 (NS)			

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Growth parameters	Altitude	Aspects (Mean $\pm$ Std. Deviation)			
		As ₁	As ₂	As ₃	Mean
Fresh shoot weight (gm)	A ₁	0.23 $\pm$ 0.12	0.24 $\pm$ 0.03	0.24 $\pm$ 0.04	0.23 ^C $\pm$ 0.06
	A ₂	0.24 $\pm$ 0.06	0.23 $\pm$ 0.03	0.21 $\pm$ 0.02	0.23 ^{BC} $\pm$ 0.04
	A ₃	0.17 $\pm$ 0.02	0.19 $\pm$ 0.03	0.20 $\pm$ 0.06	0.19 ^{AB} $\pm$ 0.03
	A ₄	0.16 $\pm$ 0.01	0.19 $\pm$ 0.02	0.17 $\pm$ 0.02	0.17 ^A $\pm$ 0.02
	Mean	0.20 $\pm$ 0.07	0.21 $\pm$ 0.03	0.20 $\pm$ 0.04	
P value _(0.05)		Alt = 0.03(S), Asp = 0.84(NS), Alt $\times$ Asp = 0.93 (NS)			
Dry shoot weight (gm)	A ₁	0.14 $\pm$ 0.07	0.13 $\pm$ 0.02	0.18 $\pm$ 0.01	0.15 $\pm$ 0.04
	A ₂	0.16 $\pm$ 0.02	0.13 $\pm$ 0.02	0.13 $\pm$ 0.02	0.14 $\pm$ 0.02
	A ₃	0.10 $\pm$ 0.01	0.07 $\pm$ 0.03	0.15 $\pm$ 0.03	0.10 $\pm$ 0.04
	A ₄	0.06 $\pm$ 0.02	0.07 $\pm$ 0.02	0.07 $\pm$ 0.02	0.07 $\pm$ 0.02
	Mean	0.12 $\pm$ 0.05	0.10 $\pm$ 0.04	0.13 $\pm$ 0.05	
P value _(0.05)		Alt = 0.40(NS), Asp = 0.48(NS), Alt $\times$ Asp = 0.21 (NS)			
Shoot/Root biomass Ratio	A ₁	1.70 ^{Aa} $\pm$ 0.61 (b)	1.65 ^{Aa} $\pm$ 0.16 (b)	2.50 ^{Ba} $\pm$ 0.67 (cd)	1.95 $\pm$ 0.62
	A ₂	1.96 ^{Aa} $\pm$ 0.15 (bc)	3.44 ^{Aa} $\pm$ 1.98 (e)	2.13 ^{ABa} $\pm$ 0.71 (c)	2.51 $\pm$ 1.26
	A ₃	2.08 ^{Ab} $\pm$ 0.25 (c)	1.33 ^{Aa} $\pm$ 0.50 (a)	2.60 ^{Bb} $\pm$ 0.23 (cd)	2.00 $\pm$ 0.63
	A ₄	2.14 ^{Aa} $\pm$ 0.47 (c)	1.81 ^{Aa} $\pm$ 1.33 (bc)	1.19 ^{Aa} $\pm$ 0.43 (a)	1.71 $\pm$ 0.84
	Mean	1.97 $\pm$ 0.39	2.06 $\pm$ 1.34	2.10 $\pm$ 0.74	
P value _(0.05)		Alt = 0.21(NS), Asp = 0.91(NS), 0.06 (S)			
Total seedling dry weight (gm)	A ₁	0.22 $\pm$ 0.09	0.21 $\pm$ 0.03	0.26 $\pm$ 0.01	0.23 ^A $\pm$ 0.05

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Growth parameters	Altitude	Aspects (Mean ± Std. Deviation)			
		As ₁	As ₂	As ₃	Mean
	A ₂	0.24 ± 0.03	0.18 ± 0.05	0.20 ± 0.03	0.20 ^A ± 0.04
	A ₃	0.14 ± 0.02	0.12 ± 0.04	0.20 ± 0.04	0.15 ^B ± 0.05
	A ₄	0.09 ± 0.03	0.12 ± 0.05	0.13 ± 0.02	0.12 ^B ± 0.04
	Mean	0.17 ^b ± 0.07	0.16 ^{ab} ± 0.06	0.20 ^a ± 0.05	
P value _(0.05)		Alt = 0.00(S), Asp = 0.09(S), Alt × Asp = 0.36 (NS)			
Survival (%)	A ₁	57.3 ± 8.33	60.0 ± 8.00	61.3 ± 4.62	59.56 ^B ± 6.46
	A ₂	61.3 ± 2.31	49.3 ± 2.31	54.7 ± 10.07	55.11 ^{AB} ± 7.42
	A ₃	58.7 ± 9.24	49.3 ± 15.14	48.0 ± 6.93	52.00 ^{AB} ± 10.77
	A ₄	45.3 ± 2.31	53.3 ± 6.11	42.7 ± 2.31	47.11 ^A ± 5.93
	Mean	55.7 ± 8.44	53.0 ± 9.05	51.7 ± 9.26	
P value _(0.05)		Alt = 0.02 (S), Asp = 0.45(NS), Alt × Asp = 0.24 (NS)			
Seedling vigor index	A ₁	895.4 ± 117.7	906.8 ± 110.50	1117.6 ± 200.57	973.28 ^C ± 168.26
	A ₂	1001.5 ± 152.4	879.4 ± 107.77	1137.5 ± 213.66	1006.14 ^C ± 180.63
	A ₃	769.4 ± 71.4	454.0 ± 37.38	884.7 ± 79.09	702.71 ^B ± 201.15
	A ₄	593.2 ± 24.4	334.1 ± 6.49	573.9 ± 47.97	500.40 ^A ± 127.90
	Mean	814.90 ^b ± 181.71	643.58 ^a ± 273.08	928.42 ^c ± 271.38	
P value _(0.05)		Alt = 0.00 (S), Asp = 0.00(S), Alt × Asp = 0.22 (NS)			
** Uppercase of alphabets in superscript used for altitude and lowercase for aspects, alphabets in parenthesis are used for overall interaction effects. Value with same alphabet means statistically similar					

Stress Condition Index

It is evident from the graph that the stress condition index increases with increasing altitude 5(a-d). The cumulative climatic stress index (SCI), which was computed by summing the proportion of dead foliage to standing foliage, the proportion of branches without leaf, and the part of the crown that was no longer living, was strongly influenced by altitudinal and aspect differences. The maximum stress condition index score was seen in the altitudinal ranges at A_4 , which was found to be substantially greater than A_1 , A_2 , and A_3 , which remained statistically equivalent. SCI was significantly lower in the south-eastern aspect in comparison to others.

Correlation Analysis

It is clear from the data presented in Figs. 6 and 7 that the cumulative effect of aspect altitude develops a highly significant positive relation with SCI only in S and NS seeds traits of *P. wallichiana*. Whereas, all other traits had an inverse relationship with altitudinal ranges. Stress condition index was found to have a significant negative relationship with viability percentage, germination capacity, germination percentage, germination speed, seedling height, collar diameter, needle area, root length, seedling dry weight, and seedling vigour index. Dark blue denotes exceptionally favorable relationships, whereas dark orange represents exceptionally negative ones.

Discussion

Germination parameters

In the present study, the impacts of altitudinal gradient, aspects, and cold stratification were discovered to have a significant influence on the seed germination characteristics of *Pinus wallichiana*. Our research is consistent with earlier studies conducted by many scientists. Significantly increased seed viability and germination metrics are observed at lower altitudes. Bhat et al. (2015) discovered that for *Pinus wallichiana* seed germination properties, a medium altitudinal range of 1600–2400 m asl is preferred. Similarly, Bhat et al. (2018) discovered variations in germination percentage, germination value (GV), and mean germination time (MGT) for *Abies pindrow* at varied altitude gradients. Between 2900 and 3200 m was the altitudinal range with the highest germination percentage (39.50%) and germination value (4.67), and the lowest germination percentage (27.25%) and germination value (1.76). Baskin et al. (1992) conducted a study on the species *Thaspium pinnatifidum* of the Apiaceae family and determined that stratification at a temperature of 5°C broke the dormancy of this species' seeds. Also, Lohengrin and Arroyo (2000) investigated the seed germination and break dormancy of *Phacelia secunda* at four heights above sea level (1600, 2200, 2900, and 3400 meters) according to cold stratification treatment, and they concluded that by increasing the height above sea level and the cooler environment, the seed germination percentage decreases with age and the time required to break dormancy increases (Spanos et al. 2012). Dorne (1981) discovered that the germination of seeds obtained from the plant

Chenopodium bonus-henricus at two elevations of 600 and 2600 meters above sea level is incomparable, with the highest germination rate occurring at 600 m altitude. According to research by Scholten et al. (2009), *Lomatium dissectum*, a member of the Apiaceae family, had a poorer germination percentage and required more time to break dormancy at an altitude of 2200 m versus 1700 m. Significant interactions were detected between latitude and stratification required in the seeds of *Pinus brutia* and seed dormancy (Thanos 2000). Studies on both physiologically dormant and non-dormant *Picea glauca* and *Picea mariana* (black spruce) seeds revealed that wet cooling enhanced the speed of germination (Wang 2000). When choosing where to harvest seeds for establishing a *Pinus wallichiana* plantation, it is recommended that the stratification of seeds, altitudinal range, and aspect be given adequate consideration.

Growth/progeny Traits

In the present study, the growth characteristics of *P. wallichiana* seedlings were found to be significantly influenced by the average effect of altitudinal ranges and by cold stratification, which is in line with the findings of other researchers in the past. Ruiz-Talonia et al. (2014) identified climatic and altitudinal patterns in seedling growth, in which seedlings from low-to-middle altitudes exhibited greater growth than populations from high elevations. The provenance of forest trees is an effective predictor of growth changes linked to environmental and climate change (Matyas 1994; Schmidting 1994). Species at higher altitudes are often adapted to colder temperatures, as evidenced by their lower growth potential, shorter times for shoot elongation, and greater resistance to cold settings compared to those at lower altitudes and in a warmer environment (Rehfeldt 1995; and Carrasquino and Goncalves 2013). The adaptive relevance of both aspects and altitudinal ranges is evident: larger seeds create more robust seedlings and larger wings permit a greater dispersal capacity (Benkman 1995; Lanner 1998). Germination traits and seed viability have been examined at the species and population levels to assess reproductive success (Mosseler et al. 2000; Mosseler et al. 2004). Dangasuk and Panetsos (2004) demonstrated that the growth performance of *P. brutia* seedlings originating from seeds collected at the middle elevation was superior to that of those originating from high and low elevations in Vorizia, confirming the genetic superiority of the population at the middle altitude. Arbez (1974) also noticed that seedlings coming from seeds collected at moderate altitudes developed quicker than those originating from low and high elevations in all examined populations. In this study, the altitudinal range (>2700 m) of *P. wallichiana* displayed inferior growth performance compared to other altitudinal gradients. This may be owing to the established growth strategy for conifers at low altitudes, which tends to be more conservative to prevent drought stress (Saenz-Romero et al. 2006). In general, provenances from higher altitudes and colder regions grew more slowly than those from warmer climates, as they may have evolved ways to endure the difficult conditions of lower winter temperatures, such as shorter growing seasons and longer and deeper dormant periods (Saenz-Romero et al. 2006 and Mitchell et al. 2013). The present study indicated a strong positive association between growth characteristics and seed vigour, which influence germination and subsequent plant growth. The results are consistent with Chauhan and Raina's (1980) findings about *P. roxburghii*. When cultivated under the same nursery conditions, seedlings from diverse

seed sources often exhibit distinct shoot growth patterns (Dormling 1979; Rehfeldt and Wycoff 1981). In this study, when seeds from different elevations were grown under identical nursery circumstances, environmental impacts should have been minimized.

Stress Condition Index

According to (Rehfeldt 1988; Sa´enz-Romero 2017), low altitude zones are connected with higher temperatures and hydric stress, whereas higher altitude zones can experience a higher frequency of frosts. These divergent environmental conditions can result in distinct genetic and phenotypic alterations among populations of certain conifer species (Saenz-Romero 2006; Viveros-Viveros, 2009; Saenz-Romero 2012]. The effect of elevation on vegetation changes has been extensively studied (Grytnes 2006; Korner 2007; Pompa-García 2013). Additionally, it was discovered in this study that the southeastern slope had healthier *P. wallichiana* trees than the southern and eastern slopes. This can be attributed to the southeastern aspect's more moderate climatic conditions than the other two. The eastern aspect of the Himalayan ecosystem receives early sun rays, exposing the trees to a fast rise in temperature following a night above freezing; whereas, trees on the southern aspect receive full sunlight during the day when the sun is quite hot.

Correlation Analysis

The correlation study of the present investigation indicated that our findings are validated by the work of previous scientists. Mughal and Thapliyal (2012) reported a significant negative correlation between seed germination and altitude ($r = -0.977$) at the 0.05 level. Seed morphological characteristics and seed germination, which serve as the foundation for selecting and distinguishing provenances, were similarly inversely linked with altitude (Mughal and Thapliyal, 2012). According to Roy et al. (2004), germination percentage ($r = -0.35$) is correlated with the latitude of the seed's origin, collar diameter has a negative correlation with latitude ($r = -0.30$) but a strong positive correlation with seed and seedling vigour. Anand and Diwedi (2014) reported a high association between biomass attributes and some morphological variables, particularly collar diameter, plant height, and leaf area, in *Bauhinia variegata*.

Conclusion

This study concludes that the germination and growth characteristics of *P. wallichiana* seedlings vary significantly based on seed source and dormancy breaking technique. All germination and growth characteristics of stratified and non-stratified seeds had the highest values at A₁ (1800–2100 m asl) and A₂ (2100–2400 m asl) elevations in the southern aspect, followed by the south-eastern side, due to the more favorable germination environment. Stratified seeds have a better germination potential than non-stratified seeds. Seeds at high elevations exhibited increased dormancy owing to the greatest climatic stress. Hence, *Pinus wallichiana* seeds must be collected at the lowermost elevation ranges, and a dormancy-breaking treatment is necessary for the vigorous development of plantations.

Declarations

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Author contributions

All authors contributed equally to the manuscript.

I declare that the manuscript has not been published in any journal/book or proceedings or any other publication, or offered for publication elsewhere in substantially the same or abbreviated form, either in print or electronically.

Conflict of interest

We have no conflicts of interest associated with this publication.

References

1. Anand RK Dwivedi SV (2014) Association studies for morphological and biomass traits of *Bauhinia variegata* Linn. Int J Agric Sci 10: 61-65.
2. Arbez M (1974) Distribution, ecology and variation of *Pinus brutia* in Turkey. FAO Forest Genetic Resources 3: 21-33.
3. Ajaz-ul-Islam, M., Masoodi, N. A., & Masoodi, T. H. (2005). Germination of blue pine (*Pinus wallichiana* Jackson) under nursery condition. J Research-Birsa Agri Uni, 17(2), 273.
4. Baskin CC, Chester EW and Baskin JM (1992) Deep complex morphophysiological dormancy in seeds of *Thaspium pinnatifidum* (Apiaceae). Int J Plant Sci 153: 565-571. <https://doi.org/10.1086/297080>
5. Baskin JM and Baskin CC (2004) A classification system for seed dormancy. Seed Sci Res 14: 1–16. <https://doi.org/10.1079/ssr2003150>
6. Bhat GM, Mughal AH, Malik AR, Khan PA, Sofi PA, Islam MA, Singh A and Shazmeen Q (2015) Altitudinal variation in different seed sources of *Pinus wallichiana* under the temperate conditions of Kashmir. The Ecoscan 9: 677-681. <https://doi.org/10.31018/jans.v9i4.1478>
7. Bhat HA, Mughal AH, Mehraj UDD and Mugloo JA (2018) Cone, seed and germination characteristics in silver fir (*Abies pindrow* Spach) along the altitudinal gradient in western Himalayas. Int J Chem Stud 6: 2052-2055.

8. Bhattacharyya A, Lamarche Jr VC and Telewski FW (1998) Dendrochronological reconnaissance of the conifers of North-West India. *Tree Ring Bulletin* 48: 21-30.
9. Bonner FT (1974) Seed testing. In: *Seeds of Woody Plants in United States*. Handbook No. 450. USDA Forest Service Washington D.C. pp.136-52.
10. Carrasquinho I and Goncalves E (2013) Genetic variability among *Pinus pinae* L. provenances for survival and growth traits in Portugal. *Tree Genet Genomes* 9: 855-66.
<https://doi.org/10.1007/s11295-013-0603-2>
11. Chauhan PS and Raina V (1980) Effect of seed weight on germination and growth of chir pine (*Pinus roxburghii* Saegent). *Indian For* 16: 53-59.
12. Czabator WM (1962) Germination value: An index combining speed and completeness of pine germination. *For Sci* 8: 386-396.
13. Dangasuk OG and Panetsos KP (2004) Altitudinal and longitudinal variation in *Pinus brutia* (Ten.) of Crete Island, Greece: some needle, cone and seed traits under natural habitats. *New For* 27: 269-284.
<https://doi.org/10.1023/b:nefo.0000022227.33131.f0>
14. Djavanshir K and Pourbeik H (1976) Germination value—A new formulat. *Silvae Genet* 25: 79-83.
15. Dormling I (1979) Influence of light and temperature on photoperiodic response of Norway spruce provenances. *Proceeding IUFRO Norway spruce Meeting Bucharest*, pp. 398-408.
16. Dorne A (1981). Variation in seed germination inhibition of *Chenopodium bonus-henricus* in relation to altitude of plant growth. *Canad J Bot* 59: 1893–1901. <https://doi.org/10.1139/b81-249>
17. Gomez KA and Gomez AA (1984) Statistical procedure for agricultural research. 2nd edition, John Wiley and Sons, New York. 357-427p.
18. Grytnes JA and Beaman JH (2006) Elevational species richness patterns for vascular plants on Mount Kinabalu, Borneo. *J Biogeogr* 33: 1838–1849. <https://doi.org/10.1111/j.1365-2699.2006.01554.x>
19. ISTA (1999) International rules for seed testing. *Seed Science and Technology, Supplement*. Zurich, Switzerland p. 27.
20. Jull LG and Blazich FA (2000) Seed germination of selected provenances of Atlantic white cedar as influenced by stratification temperature and light. *Sci Hortic* 35:132-135.
<https://doi.org/10.21273/hortsci.35.1.132>
21. Korner C (2007) The use of “altitude” in ecological research. *Trends Ecol Evol* 22: 569–574.
22. Lohengrin A, Cavieres and Mary TKA (2000) Seed germination response to cold stratification period and thermal regime in *Phacelia secunda* (Hydrophyllaceae). *Plant Ecol*: 1-8.
23. Lopez T, Heredia MH, Castellanos DA, Garcia AB and Romero CS (2017) Reproductive investement of *Pinus pseudostrobus* along an altitudinal gradient in Western Mexico: implications of climate change. *New For* 10: 1056-1060. <https://doi.org/10.1007/s11056-017-9602-8>
24. Matyas C (1994) Modeling climate change effects with provenance test data. *Tree Physiol* 14: 797-804. <https://doi.org/10.1093/treephys/14.7-8-9.797>

25. Mitchell RG, Wingfield MJ, Hodge GR, Dvorak WS and Coutinho TA (2013) Susceptibility of provenances and families of *Pinus maximinoi* and *Pinus tecunumanii* to frost in South Africa. *New For* 44: 135-146. <https://doi.org/10.1007/s11056-012-9306-z>
26. Mosseler A, Major JE, Simpson JD, Daigle B, Lange K, Park YS, Johnsen KH and Rajora OP (2000) Indicators of population viability in red spruce, *Picea rubens*. I. Reproductive traits and fecundity. *Canad J Bot* 78: 928–940. <https://doi.org/10.1139/b00-065>
27. Mosseler A, Rajora OP, Major JE and Kim KH (2004) Reproductive and genetic characteristics of rare, disjunct pitch pine populations at the northern limits of its range in Canada. *Conserv Genet* 5: 571–583. <https://doi.org/10.1007/s10592-004-1850-4>
28. Mughal AH and Thapliyal RC (2012) Provenance variation in cone and seed characteristics of *Cedrus deodara* (D. DON) (G. DON) in Jammu and Kashmir. *J For Res in China* 14: 193-199. <https://doi.org/10.1007/s11632-012-0306-z>
29. Pattanaik, S., Dash, A., Mishra, R. K., Nayak, P. K., & Mohanty, R. C. (2015). Seed germination and seedling survival percentage of *Shorea robusta* Gaertn. f. in buffer areas of Similipal biosphere reserve, Odisha, India. *J of Ecosystem & Ecography*, 5(1), 1-1.
30. Pompa-Garcia M, Cerano-Paredes J and Fule´ PZ (2013) Variation in radial growth of *Pinus cooperi* in response to climatic signals across an elevational gradient. *Dendrochronologia* 31: 198–204. <https://doi.org/10.1016/j.dendro.2013.05.003>
31. Rehfeldt GE and Wycoff WR (1981) Periodicity in shoot elongation among populations of *Pinus contorta* from the northern Rocky Mountain. *Ann Bot* 48: 371–377. <https://doi.org/10.1093/oxfordjournals.aob.a086135>
32. Rehfeldt GE (1988) Ecological genetics of *Pinus contorta* from the Rocky Mountains (USA): a synthesis. *Silvae Genet* 37: 131–135
33. Rehfeldt GE (1995) Genetic variation, climate models and the ecological genetics of *Larix occidentalis*. *For. Ecol. Manag* 78: 21-37. [https://doi.org/10.1016/0378-1127\(95\)03602-4](https://doi.org/10.1016/0378-1127(95)03602-4)
34. Roy MS, Thapliyal RC and Phartyal SS (2004) Seed source variation in cone, seed and seedling characteristics across the natural distribution of Himalayan low level pine *Pinus roxburghii*- sarg. *Silvae Genet* 53: 116-122. <https://doi.org/10.1515/sg-2004-0021>
35. Ruiz-talonia LF, Sánchez-vargas NM, Bayuelo-jiménez JS, Lara-cabrera SI and Sáenz-romero C (2014) Altitudinal genetic variation among native *Pinus patula* provenances: performance in two locations, seed zone delineation and adaptation to climate change. *Silvae Genet* 63: 139-149. <https://doi.org/10.1515/sg-2014-0019>
36. Sa´enz-Romero C, Larter M, Gonza´lez-Mun˜oz N, Wehenkel C, Blanco-Garcia A, Castellanos-Acun˜a D, Burlett R, Delzon S (2017) Mexican conifers differ in their capacity to face climate change. *Journal of Plant Hydraulics* 4: 003-006. <https://doi.org/10.20870/jph.2017.e003>
37. Saenz-Romero C, Gzman-Reyna RR and Rehfeldt GE (2006) Altitudinal genetic variation among *Pinus oocarpa* populations in Michoacan, Mexico. *For Ecol Manag* 229: 340-350. <https://doi.org/10.1016/j.foreco.2006.04.014>

38. Saenz-Romero C, Rehfeldt GE and Soto-Correa JC (2012c) Variación genética altitudinal entre poblaciones de *Pinus pseudostrobus* de Michoacán, México: resultados de ensayo en casas de sombra en dos localidades. *Rev Fitotec Mex* 35: 111–121.
39. Schmidting RC (1994) Use of provenance tests to predict response to climate change: loblolly pine and Norway spruce. *Tree Physiol* 14: 805–817. <https://doi.org/10.1093/treephys/14.7-8-9.805>
40. Scholten M, Donahue J, Shaw NL and Marcelo D (2009) Environmental regulation of dormancy loss in seeds of *Lomatium dissectum* (Apiaceae) *Ann Bot* 103: 1091–1101. <https://doi.org/10.1093/aob/mcp038>
41. Spanos I, Platis P and Meliadis I (2012) Comparative study among experimental plantings and phenotypes of cork oak (*Quercus suber* L.), Northern Greece: 18 years later. *Int J For Soil Eros* 2 (2): 78-84.
42. Thanos CA (2000) Ecophysiology of seed germination in *Pinus halepensis* and *P. brutia*. In: *Forest ecosystems in the Mediterranean basin* (Eds.: Ne'eman G. and Trabaud, L.), Backhuys Publishers, Leiden. pp. 37- 50. <https://doi.org/10.1006/anbo.2000.1313>
43. Venable DL (1984) Using intraespecific variation to study the ecological significance and evolution of plant life-histories. Pp 166–186. In: Dirzo, R. & Sarukhán, J. (eds) *Perspectives on Plant Population Ecology*. Sinauer, Sunderland. <https://doi.org/10.1126/science.227.4685.405>
44. Viveros-Viveros H, Saenz-Romero C and Vargas-Hernández JJ (2009) Altitudinal genetic variation in *Pinus hartwegii* Lindl. I: height growths shoot phenology, and frost damage in seedlings. *For Ecol Manag* 257: 836–842. <https://doi.org/10.1016/j.foreco.2008.10.021>
45. Viveros-Viveros H, Saenz-Romero C, Upton JL and Hernandez JV (2005) Variación genética altitudinal en el crecimiento de plantas de *Pinus pseudostrobus* Lindl. en campo. *Agrociencia* 39: 575–587. <https://doi.org/10.35196/rfm.2005.4.333>
46. Wang BSP, Berjak (2000) Beneficial Effects of Moist Chilling on the Seeds of Black Spruce (*Picea mariana* [Mill.] B.S.P.) *Ann Bot* 86(1): 29-36. <https://doi.org/10.1006/anbo.2000.1150>

Figures

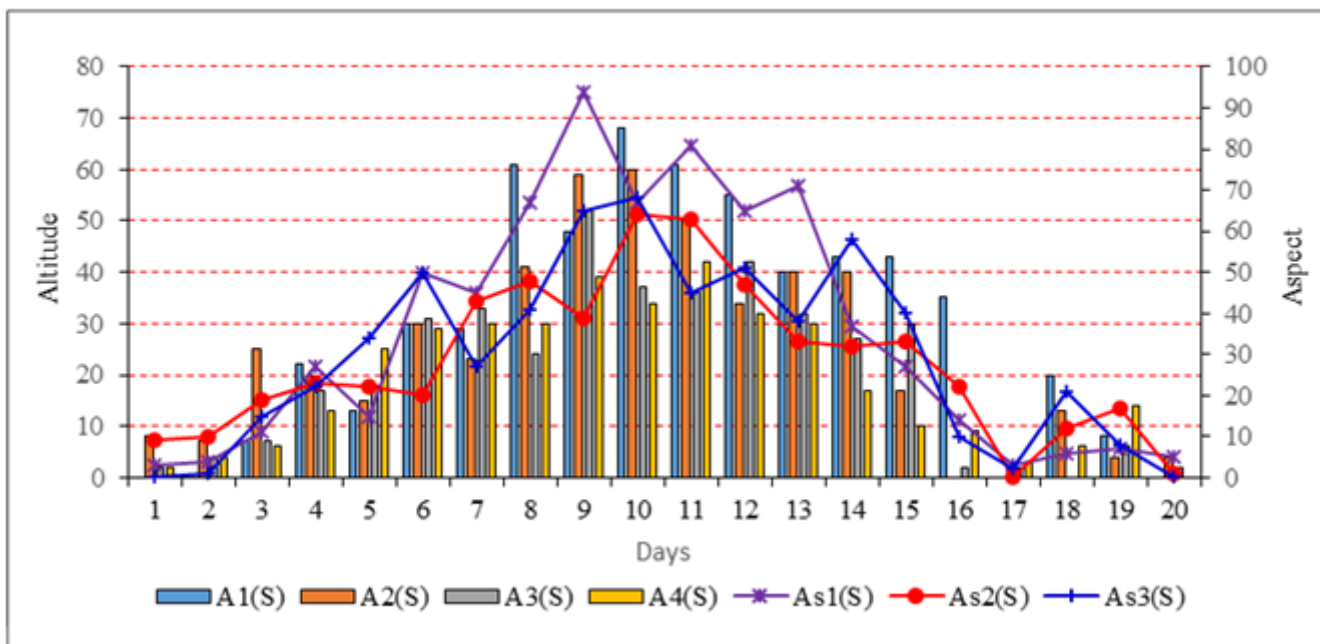


Figure 1

Daily germination of stratified seeds of *P. wallichiana* collected from different altitudes and aspect in nursery

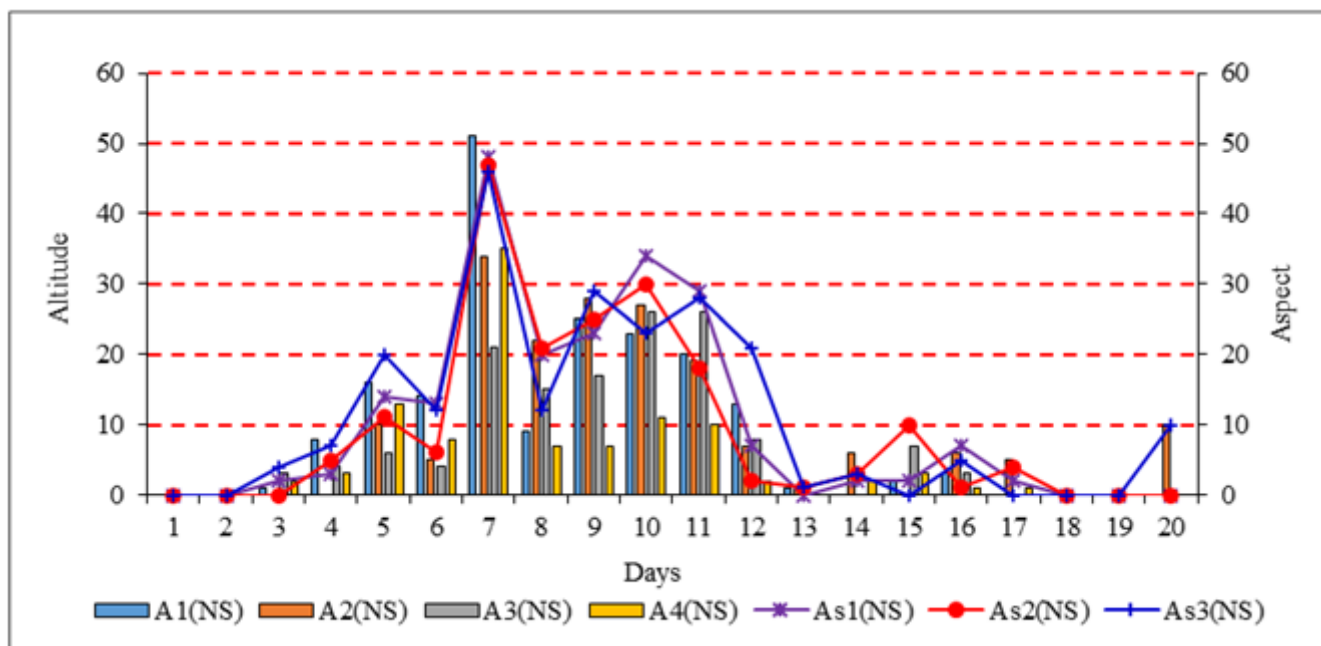


Figure 2

Daily germination of non-stratified seeds of *P. wallichiana* collected from different altitudes and aspect in nursery

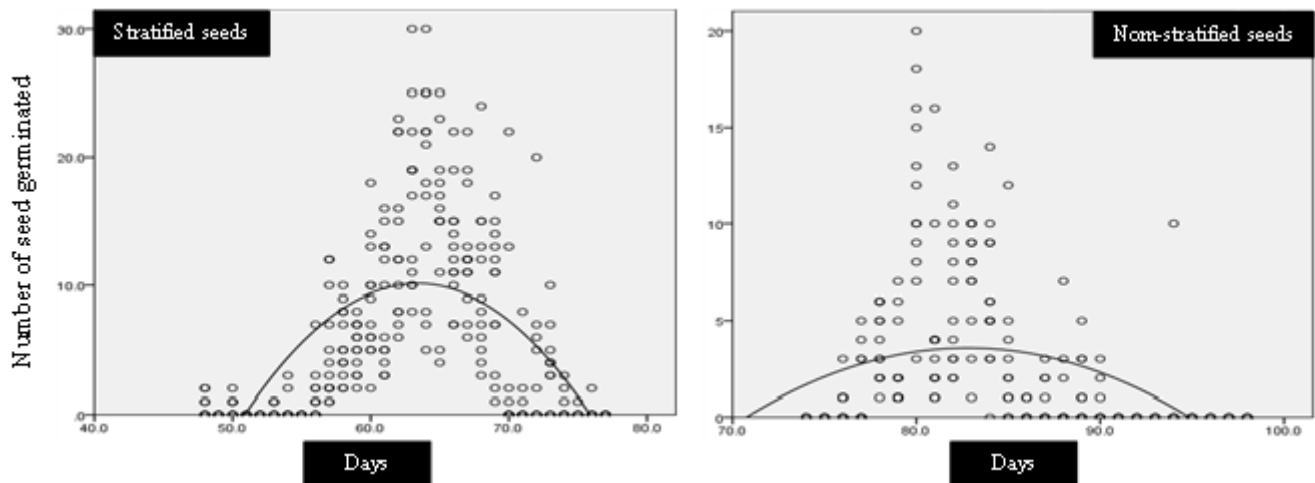


Figure 3

Number of seeds germinated of *P. wallichiana* after dormancy breaking treatment

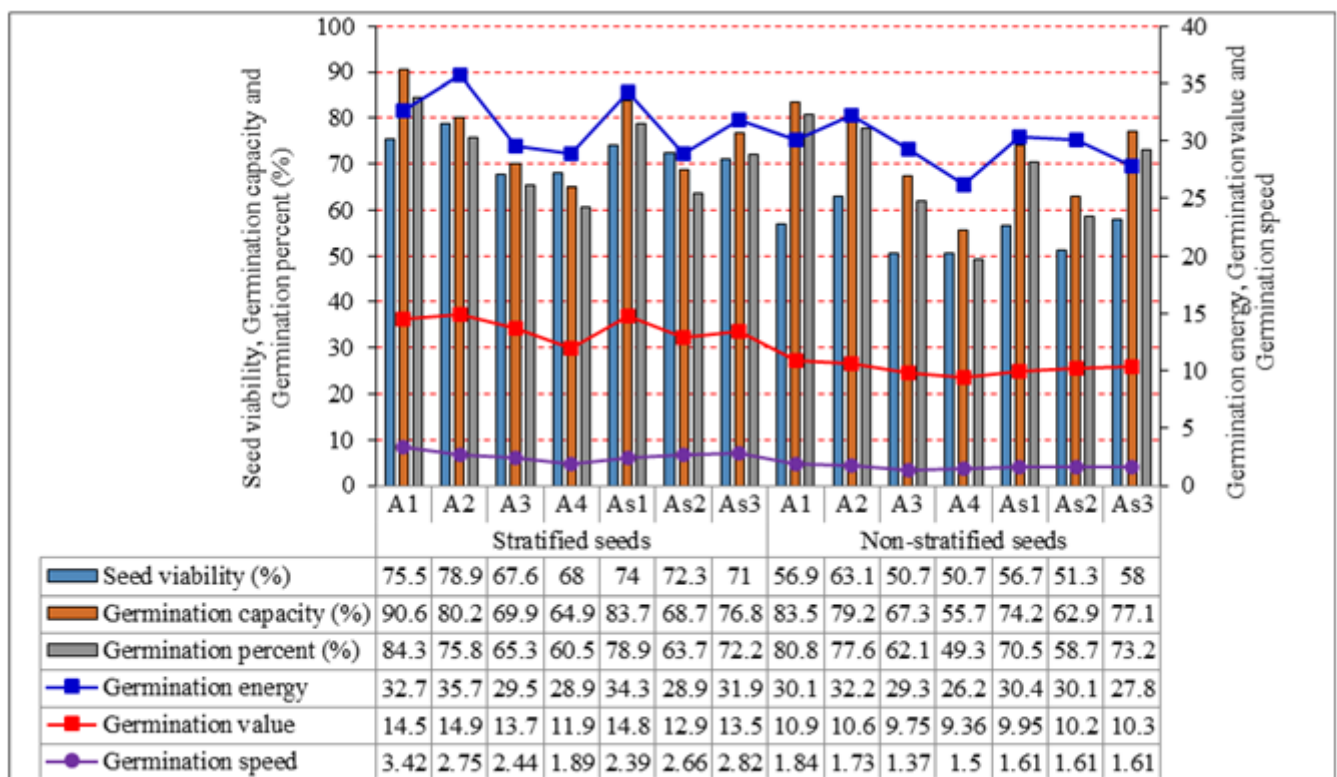


Figure 4

Effect of altitudinal ranges, and aspects on stratified and non-stratified seeds germination of *P. wallichiana*

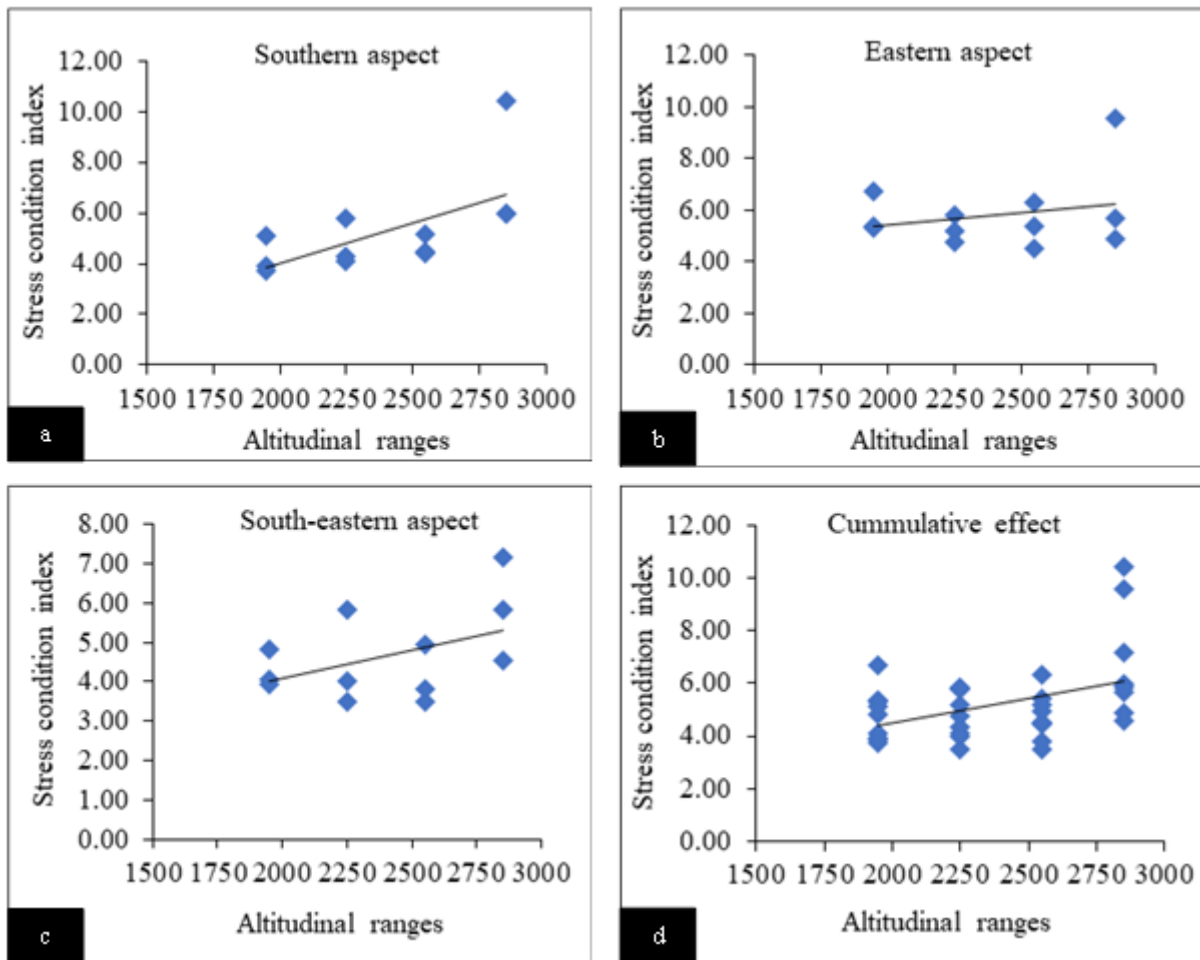


Figure 5

(a-d) Relations between of altitudinal ranges and stress condition index (SCI) of *P. wallichiana* trees in different aspect

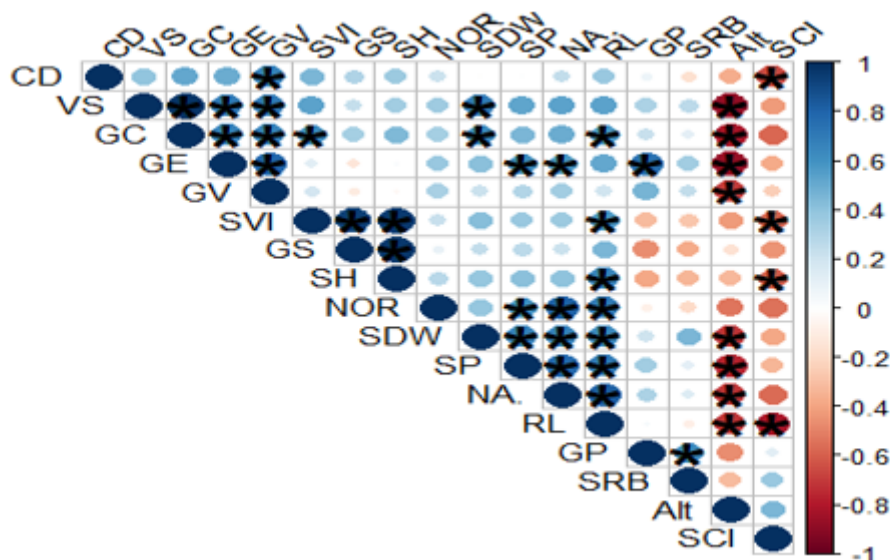


Figure 6

Correlation coefficients (r) between altitude with germinability and growth of stratified seeds w.r.t. different aspects

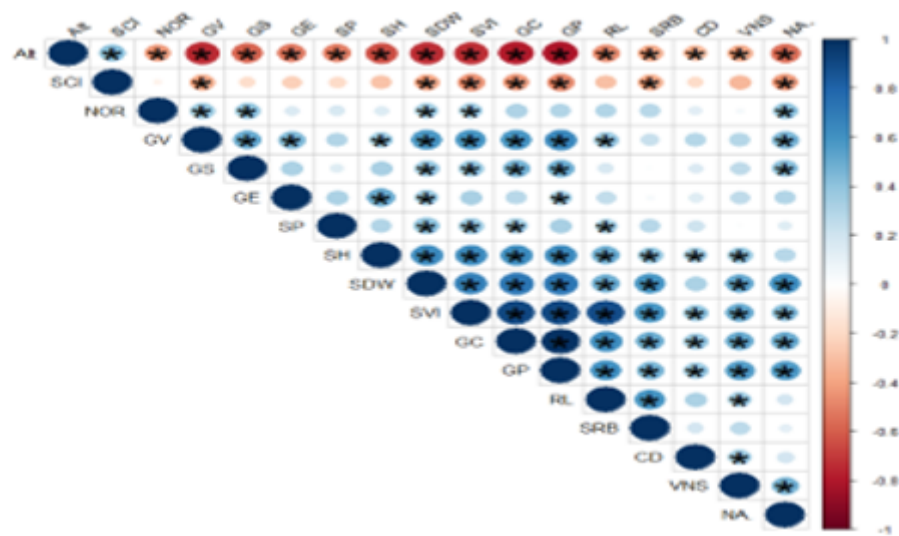


Figure 7

Correlation coefficients (r) between altitude with germinability and growth of non- stratified seeds w.r.t. different aspects

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