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# Seed source variation and conservation of *Pinus wallichiana* in India

**M. Thapliyal, O. Singh, B. Sah, N. Bahar**

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Abstract. *Pinus wallichiana* A.B. Jacks is an important component of the middle and high altitude Himalayan forests having large natural distribution ranging between $26^0$ to $36^0$ N latitude and $69^0$ to $75^0$ E longitudes. It is commonly known as Himalayan blue pine or blue pine, being indigenous to Himalayan Mountain regions and because of its bluish or grayish-green leaves. It is a five needle pine which gained world-wide attention for its resistance to blister rust among white pines. This species has been crossed successfully with other white pines and vigorous hybrids have been obtained. Considerable variation in morphological and anatomical characteristics of needles, cones and seeds in natural stands exists across the natural distribution of the species, especially in mesic and xeric habitats. These variations suggested the differentiation of this species in ecotypes or varieties as reported by various authors. However, the level of genetic diversity was found to be relatively high and the degree of genetic differentiation was low compared to other pines. The wide range of climatic conditions in the natural distribution of this pine is expected to result in high genetic variation within different populations of the species. The study aims to determine the nature and extent of variation present in the populations of the species in respect to cone and seed characteristics across its natural distribution. Seed of 17 seed sources from the states of Uttaranchal and Himachal Pradesh was collected and analyzed for cone characters (fresh weight of cones, cone length, cone width, specific gravity) and seed characters (seed weight, moisture content, germination percent, cotyledon number). Significant variations have been observed in these traits among different seed sources of the species. The cone weight varied from 44.4 to114g and the higher cone weight was recorded at higher altitudes. The germination percent varied from 40 to 85 whereas cotyledon number varied from 7 to12. Seed weight ranged from 35.70 to 69.30g in all seed sources. These variations can be utilized further in identifying the best seed sources in improving the productivity of the species. The genetic variability present in this pine has been conserved in preservation plots demarcated and maintained in its natural ranges of distribution in the country, since the early 19th century

Keywords: *Pinus wallichiana,* seed source, variation, cone, seed, conservation

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## Introduction

Geographical variation exists in almost all the species of flora and fauna. The differences in growth and development processes of the species have taken a long time to evolve over many types of agroclimatic conditions. The knowledge of genetic variations within the species is essential for developing effective

tree improvement and breeding strategies, which starts with the scanning of available variation in the distribution range of the species and delineation of seed zones/region of provenance capable of producing the best adaptable trees (Callaham 1964, Wright 1976). Variation within populations of tree species has been exploited in the selection of superior seed source/provenance for a given site and for evolving conservation strategies of genetic diversity within populations of tree species (Rawat et al. 2006).

Studies on source variation in seed and seedling traits have been conducted on numerous species of Pines, such as *Pinus banksiana* Lamb (Yeatman 1966); *Pinus caribaea* Mor. (Venator 1974); *P. brutia* (Ishik 1986); *P. elliottii* var. densa (Bethune & Lanngdon 1966); *P. greggii* Engelm. (Dvorak et al. 1996); *P. roxburghii* (Mukherjee 2003) etc. Pines constitute one of the most divergent and economically important forest tree species and account for as much as 70% of all woodland areas (Shurkhal et al. 1992).

*Pinus wallichiana*, commonly known as blue pine, is a tall straight evergreen tree (PARKER 1956). It is a five needle pine, which gained world-wide attention for its resistance to blister rust among white pines. From a timber point of view, blue pine is considered next only to deodar (*Cedrus deodara*) among conifers. Considerable variation in morphological and anatomical characteristics of needles, cones and seeds in natural stands exists across the natural distribution of the species, especially in mesic and xeric habitats (CABI 2002). Blue pine has a large natural distribution and is an important component of the middle and high altitude Himalayan forests. The longitudinal and latitudinal distribution of this species is 68 to $100^0$ East and 25 to $36^0$ North, respectively. Occurring between 1500 to 3800m altitudes, this species is extensively found in Afghanistan, Pakistan, India, Nepal, Bhutan, Myanmar and China (Troup 1921, Critchfield & Little 1966). Branches are whorled, spreading, usually ascending in young trees and horizontal or drooping with upturned ends in old trees. Two whorls are often produced in one year (Troup 1921).

### Screening of seed sources

Dogra (1972) described seven broad provenance types in this species, four-high and low level types in the inner dry and outer monsoon regions in the western Himalayas and inner dry, middle moist and outer wet types in the eastern Himalayas. Kriebel & Dogra (1986) carried out a seed source trial of this species in Ohio and reported significant intra-specific variation. Dogra suggested further investigations using seed sources from its extensive range of distribution, hence the present investigations. Studies of the seed biology of *Pinus wallichiana* have been done on a very limited scale (Thapliyal et al. 1985). The occurrence of blue pine over a wide geographic range encompasses a great diversity of edapho-climatic conditions in its habitat, which is expected to be reflected in the genetic constitution of its population, thereby offering an opportunity to study variation. Despite this, information available on basic genetics or geographic variation in cone and seed traits of blue pine is scanty and insufficient. The present endeavor therefore, is towards studying and assessing the pattern and magnitude of geographic variation among different seed sources of the Himalayan blue pine to help understand natural patterns that may also exist in genetic variation and adaptation. These patterns could be useful to select genetically suitable material could be screened out for plantation use.

## Material and methods

The mature cones of *Pinus wallichiana* were collected from 17 different sources within the natural distribution ranges of the species from the Himalayan states of Himachal Pradesh and Uttaranchal (table 1). Cones were collected from 10 randomly selected average trees located at least 100m apart from each other at each of the location. The laboratory work was carried out at the Forest Tree Seed Laboratory, Forest Research Institute, Dehradun.

### Cone Characteristics

Cones were cleaned of twigs, bark, foliage and

other impurities before drying (figure 1) for seed extraction. The selected 20 cones were dried in the laboratory at a temperature of 45$^0$C, whereas the rest of the cones were sun-dried (figure 2) and the seeds extracted (figure 3) from them, cleaned, dewinged and stored at 15$^0$C/15% RH in sealed containers.

Measurements were recorded for cone size (length, width), fresh weight (figure 1) and specific gravity of cone and number of seeds per cone using twenty randomly drawn mature cones from each location. Cone length and width was measured using electronic calipers. Specific gravity of 10 mature closed cones was measured by using displacement method (Oliver 1974).

## Seed biology

Seed Size: The seed dimensions i.e. seed length and seed width of 25 seeds were recorded in millimeters using electronic calipers. Seed weight: Seed weight was taken of the pure seed fraction in replications and expressed as the 1000 seed weight.

Moisture content: Percent moisture content of the seeds was recorded on wet basis at 103$^0$C for 17 hours, in four replications (ISTA 1993).

Seed Germination. Germination in *P. wallichiana* is epigeous. The radicle emerges

**Table 1** Geographical position and altitude of 17 seed collection localities of *Pinus wallichiana.*

| Sl. no. | Seed Source | State | Longitude ($^0$E) | Latitude ($^0$N) | Altitude (m) |
|---|---|---|---|---|---|
| 1 | Panthaghati | Himachal Pradesh (H.P.) | 77.36 | 31.10 | 2400 |
| 2 | Kufri | H.P. | 78.23 | 30.15 | 2622 |
| 3 | Theog | H.P. | 78.55 | 30.05 | 2635 |
| 4 | Kandiali | H.P. | 78.87 | 29.65 | 2640 |
| 5 | Kotgarh | H.P. | 7831 | 31.50 | 2750 |
| 6 | Nichar | H.P. | 78.15 | 31.35 | 2768 |
| 7 | Sarahan | H.P. | 77.57 | 32.03 | 2800 |
| 8 | Kumarsain | H.P. | 78.53 | 31.05 | 2532 |
| 9 | Jagat Sukhi | H.P. | 78.08 | 31.16 | 2250 |
| 10 | Nathan | H.P. | 77.48 | 31.48 | 2600 |
| 11 | Naggar | H.P. | 77.69 | 31.05 | 2585 |
| 12 | Chamba | H.P. | 76.32 | 32.26 | 2870 |
| 13 | Gangotri | Uttaranchal (UA) | 77.34 | 28.53 | 3140 |
| 14 | Barkot | UA | 78.14 | 30.48 | 1524 |
| 15 | Nandprayag | U A | 79.50 | 30.70 | 762 |
| 16 | Joshimath | U A | 79.53 | 30.24 | 3049 |
| 17 | Nagnath | U A | 79.23 | 30.15 | 1615 |

**Figure 1** Green cone of *P. wallichiana*

**Figure 2** Mature cone of *P. wallichiana*

(figure 4) from the end of the seeds and descends, the hypocotyl elongates and sometimes arches slightly and the cotyledons, 7-12 in number are carried above the ground. As the cotyledons elongate the shell of the seed remains, enclosing their apices for some time, eventually falling to the ground, when the cotyledons spread out radially in a whorl, from the centre of which the young shoot develops. Seeds were germinated on Petri dishes on moist blotters at $25^0$C, in 4 replications of 50 seeds each.

Seedling parameters i.e. root length, shoot length and cotyledon number were recorded when the germination had completed. The root and shoot length of 20 seedlings were measured in centimeters with the help of a meter scale.

## Results and discussion

Cone traits as well as seed traits revealed significant differences among seed sources at 5% level of significance (table 2). Variation was observed in the fresh weight of cones, cone length and cone width among the seed sources (table 2). The cone fresh weight varied from 44.4g in Panthaghati (HP) source to 114g at Kandiali (HP) source. Maximum cone length and width was recorded at 22.80 cm at Jagat Sukhi (HP) and 39.29 mm at Kandiali (HP) while minimum length and width were recorded at 12.94 cm and 21.10 mm both at Panthaghati (HP). Such types of variation in cone characters of *P. wallichiana* was also observed by Singh et al. (1996).

Specific gravity as an index of maturity has been established for cones of a number of conifer species. The cone to be tested is placed in a liquid in which it will float if mature and sink if immature. Maximum specific gravity of 0.85 was for the Sarahan seed source while the minimum was 0.71 for the Kufri seed source.

Seed traits such as, seed length, width, weight and moisture content varied significantly among seed sources. Seed size may vary due to both internal (maternal, hereditary) and external (environmental) conditions operating at the time of seed development (Harper et al. 1970). Variation in *Pinus wallichiana* seed sources with respect to their morphological characters could be due to the fact that this species grows over a wide range of climatic conditions. In the case of seed dimensions i.e. seed length and seed width, however, very little variation was found. Maximum and minimum seed length were recorded at 9.22 and 6.94 mm respectively, for Kotgarh and Jagat Sukhi (HP) sources while maximum and minimum seed width was recorded at 7.73 and 4.43 mm for Nandprayag (UA) and Theog (HP) sources, respectively.

Seed size is a major trait in agriculture-crop improvement, since it is under strong genetic influence (Fehr & Weber 1968). A positive correlation of seed size with seedling size was observed in several pines (Spurr 1944).

Seed Weight: The consideration of seed weight in the delineation and understanding of the geographical variation has been advocated because of the low plasticity of this character (Harper et al. 1970) as it is mainly influenced by maternal factors and is under strong genetic control (Lindgren 1984 & Tyson 1989). Vakshasya et al. (1992) observed that seed weight is an important variable in selecting and delineating seed sources and indicated the

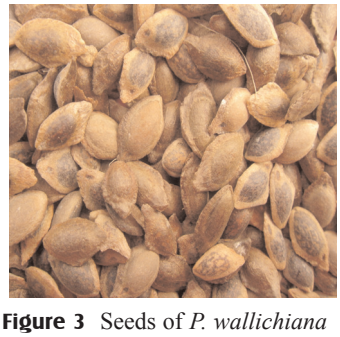

**Figure 3** Seeds of *P. wallichiana*

**Figure 4** Seed germination stages of *P. wallichiana*

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

| Sl. no. | Parameters | | Range | Mean | SD | CV% | CD (Level 0.05) |
|---|---|---|---|---|---|---|---|
| 1. | Cone Weight | (g) | 44.4- 114 | 75.59 | 17.82 | 23.57 | 25.84 |
| 2. | Cone Length | (cm) | 12.9- 22.8 | 18.46 | 2.51 | 13.57 | 3.59 |
| 3. | Cone Width | (cm) | 21.1- 39.29 | 30.98 | 4.41 | 14.24 | 6.29 |
| 4. | Seed Length | (mm) | 6.94 - 9.22 | 7.92 | 0.56 | 7.06 | 0.65 |
| 5. | Seed Width | (mm) | 4.43 - 7.73 | 4.90 | 0.76 | 15.47 | 0.49 |
| 6. | 1000 Seed Weight | (g) | 35.7- 69.3 | 48.23 | 9.18 | 19.00 | 4.84 |
| 7. | Moisture content | (%) | 7 - 23.7 | 12.66 | 4.63 | 36.61 | 3.16 |
| 8. | Lab Germination | (%) | 40- 85 | 57.71 | 12.12 | 21.01 | 21.20 |
| 9. | Root Length | (cm) | 3.6 - 7.35 | 4.91 | 0.93 | 18.92 | 0.93 |
| 10. | Shoot Length | (cm) | 4.25 - 7.1 | 5.72 | 0.93 | 16.33 | 1.10 |
| 11. | Cotyledon number | | 7- 12 | 10 | 0.81 | 7.9 | 1.39 |

possibility of the existence of dry and wet zone populations.

Maximum seed weight recorded was 69.30g for Kandiali (HP) seed source and the minimum was 35.70g for Naggar (HP) source.

Moisture content: When the seed has reached its full size, and as the embryo approaches maturity, a very rapid moisture loss occurs in many species. Shortly after fertilization the endosperm and embryo of sugar pine (*Pinus lambertiana* Dougl.) contain as much as 70 percent moisture. At the time of natural seed dispersal, moisture content of the pine embryo is reduced to 30 percent and in the female gametophyte it is reduced to about 60 percent (USDA 1974). A further reduction in moisture content down to 6-12 percent is desirable to maintain viability of many species of seeds in storage. 23.7% moisture content was the recorded maximum at Kandiali (H.P.) and 7% minimum at Nagnath (UA). Highest coefficient of variability, 36.61%, was observed in moisture content as the seed collected from various sources was at different stages of maturity.

Since the seeds were collected from different locations, from trees approximately of the same age, differences observed in seed parameters may be attributed to the different architectures developed as a result of adaptation to diverse environmental conditions prevailing throughout their distributional range (Salazar & Quesada 1987).

Germination percentage of *P. wallichiana* seeds showed a wide variation within provenances of Himachal Pradesh and Uttaranchal. A maximum of 85% germination was recorded at Kandiali (HP) and a minimum of 40% at Nandprayag (UA). Seed weight positively correlated with seed germination percent as filled and heavier seeds result in high germination as was observed in the Kandiali seed source for which the highest values for both the parameters were recorded. 21.01% coefficient of variability was recorded in germination as the seeds in some sources were immature when collected, which resulted in lower germination in such seed sources.

Seedling parameters i.e. root length, shoot length and cotyledon number showed very little variation among different provenances of *P. wallichiana*. Maximum root length was 7.35 cm at Joshimath (UA) while 3.6 cm was the minimum at Nathan (HP). 7.10 mm maximum shoot length was recorded at Naggar (HP) while the minimum was 4.25 mm at Theog (HP). Length of the root and shoot measured at the end of the experiment are an indicator of the vigour of the seeds and speed of growth of the seedlings. Cotyledon numbers varied from 7-12.

Highest coefficient of variation was recorded for moisture content of seed (36.61) followed by 23.57 for cone weight and then 21.01 for laboratory germination of seeds. The differences recorded may be in response to different intensities of natural selection pressure acting upon these traits in their natural habitat.

## Conservation of *Pinus wallichiana* in India

Much of the natural environment has been altered to varying degrees since man started using the natural resources of the earth. From the earliest times, unrestricted fellings, fire,

grazing and shifting cultivation have been responsible for degeneration of many habitats and consequent extinction or near extinction of many plant species. This is particularly true for many Asian and African countries including India, where both the pace and impact of developmental activities are more pronounced. The need of the day is for better living and prosperity for the people with continued availability of the resource. This is in consonance with the definition of conservation, 'rational use of earth's resources'. Forests have been were demarcated as protected natural areas where, by legal restrictions, natural conditions are maintained and natural ecological processes are allowed to predominate keeping human interference to a minimum. The 'preservation plots' were established in India as early as 1905-1906 and consequently more plots were preserved on the recommendations of the All India Silvicultural Conferences. The sole objective of such plots was to have a fine specimen of a forest, a group of outstanding trees, to preserve patches of well grown forests. The extent of such areas varied from 0.01 ha to 4000 ha. *Pinus wallichiana* being a very important timber species of the temperate forest, its preservation plots have also been demarcated in pure and mixed patches with *Cedrus deodara* as well as *Abies pindrow*. These plots were created with the objective of observing natural succession, for which they had to be protected from all types of injury to permit normal progression towards the climax form, removal of dead, uprooted or badly damaged trees, at times permitting some green felling to ensure that the plot remains true to the type it represents, and that germplasm of exceptional trees thrives in healthy conditions as long as possible, for posterity. Germplasm can be utilized for tree improvement programmes of the species, for enhancing productivity and disease resistance.

## Conclusions

There were significant differences in cone and seed traits between the seed sources. This geographic variation may be useful in guiding selection of the most promising sources for further improvement and breeding programmes. However, such studies need to be done on a wider scale, i.e. on seed sources all across the natural distribution range of the species and also at the stage of nursery emergence and performance.

The white pines in the United States are highly susceptible to blister rust caused by *Cronartium ribicola* J.C. Fisch ex Rabenh. *P. wallichiana* crosses with other white pines have produced hybrids resistant to blister rust, which show adaptability, good growth and hybrid vigour. The good seed set seen in some of the inter-specific cross-combinations with this species makes large-scale production of hybrids possible. The germplasm of blue pine used in crosses with other white pine species earlier was of unknown geographic origin. Information on the different provenances of *P. wallichiana* growing in the Himalayas will, therefore, be useful to tree breeders in planning provenance tests and breeding strategies for productivity and disease resistance. The germplasm of the species conserved in preservation plots can be utilized for the same. Blue pine is not planted presently as there are other important tropical timber species, including the three-needle pines, but it may be a major plantation species in the future. Provenance research for the delineation of seed zones and selection of better and well-adapted seed sources/provenances will help in a big way with efforts towards reforestation and restoration.

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**Rezumat.** Thapliyal M., Singh O., Sah B., Bahar N., 2008. variaţia surselor de seminţe la *Pinus wallichiana* şi conservarea în India. Ann. For. Res. 51: 81-88.

*Pinus wallichiana* A.B. Jacks este un component principal al pădurilor naturale de altitudine medie şi mare din Himalaya a căror areal este cuprins între $26^0$ şi $36^0$ latitudine nordică şi $69^0$ şi $75^0$ longitudine estică.

Este o specie cu cinci ace de culoare albăstruie, care pentru rezistenţa sa la rugina veziculoasă s-a bucurat de mare atenţie. Această specie a fost încrucişată cu succes cu alţi pini obţinându-se hibrizi viguroşi.

Pretutindeni în arealul natural al speciei există o mare variabilitate în privinţa caracteristicilor anatomice şi morfologice ale acelor, conurilor şi seminţelor îndeosebi în habitatele situate în climat moderat şi excesiv uscat.

Potrivit diferiţilor autori, această variabilitate sugerează diferenţierea speciei în ecotipuri sau varietăţi. Cu toate acestea, gradul de diferenţiere genetică a fost mic iar nivelul diversităţii genetice relativ ridicat comparativ cu alţi pini. Este de aşteptat ca variabilitatea condiţiilor climatice din arealul natural al acestui pin să aibă ca rezultat o mare variabilitate genetică în interiorul diferitelor populaţii.

Scopul studiului a constat în determinarea naturii şi amplitudinii variaţiei caracteristicilor conurilor şi seminţelor prezente în populaţiile studiate din arealul natural. Şaptesprezece populaţii aparţinând statelor Uttaranchal and Himachal Pradesh au fost analizate în privinţa unor caractere ale conurilor (greutatea conului proaspăt, lungimea, grosimea, greutatea specifică) şi sem-

inţelor (greutatea seminţei, conţinutul în umiditate, procentul de germinaţie, numărul cotiledoanelor).

Între diferite origini au fost puse în evidenţă variaţii importante ale caracterelor studiate ale speciei. Masa conului a variat între 44.4 şi 114 g cu menţiunea că greutatea conului cea mai mare a fost înregistrată la altitudini mai înalte. Procentul de germinaţie a variat între 40 şi 85 în timp ce numărul de cotiledoane se înscrie în intervalul de la 7 la 12. Masa a 1000 seminţe a fost cuprinsă în intervalul de 35,7 şi 69,3g. Aceste variaţii pot fi utilizate în viitor la identificarea celor mai valoroase surse de seminţe necesare pentru ameliorarea productivităţii speciei. Variabilitatea prezentă în acest pin a fost conservată in situ încă de la începutul secolului al 19-lea.

**Cuvinte cheie**: *Pinus wallichiana*, surse de seminţe, variaţie, con, sămânţă, conservare.

(Tradus de I. Blada)