ENVIRONMENTAL VARIATION IN SEED AND SEEDLING CHARACTERISTICS OF *PINUS ROXBURGHII* SARG. FROM UTTARAKHAND, INDIA

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Abstract. The present study was undertaken to assess the environmental variation in seed characters and to explore the efficacy of hydrogen peroxide treatment on the germination and seedling traits of sixteen provenances of Pinus roxburghii from Uttarakhand Himalaya. Provenances, which had higher values for seed parameters showed better germination. Soaking the seeds for 24 hours in a solution of H2O2 (1% v/v) had a significant effect on the rate of germination and average germination percentage. About 88.5 % of mean average germination was revealed by the seeds that were treated with H2O2 as compared to the untreated (control) seeds (77.4 %). Simultaneously, H2O2 treatment also caused an appreciable decrease, in shortening of germination period by 10 days. Results have shown that the soaking of chir-pine seeds in H2O2 (1% v/v) for 24 hours is highly beneficial and therefore for large scale germination, treatment of H2O2 should be preferred. The germination percentage of seeds was found to be positively correlated with altitude and negatively correlated with rainfall.
Keywords: *Garhwal Himalaya, Provenance, Altitude, Hydrogen per oxide treatment, Seedling height.*

Introduction

The natural regeneration of different plant species through seeds depends upon the production and germination capacity of the seeds and the successful establishment of the seedlings. Seeds from healthy, well formed trees provide greater assurance that the resulting stock will have good form, survival and resistance against stress conditions. A tree species may extend over the large geographical area and can grow in wide range of environments with varying climate and topography. It usually has local populations adapted to the different environment at conditions, known as provenances. After knowing the range of adaptation of a species its populations may become more important in forest restoration, as global warming and other environmental changes disrupt natural forests (Ledig & Kitzmiller, 1992). In provenance trials, provenances from varying climatic conditions are compared to asses the seed quality. As an early step towards improvement of tree species, provenance trials provide prerequisite knowledge of both genetic and environmental variations, which allow direct genetic comparisons among seed sources growing in multiple “common gardens” and are ideally suited to quantify impacts of changing climate on wood production (Kitzmiller, 2005).

Pinus roxburghii Sargent, commonly known as ‘Chir pine’ is the most important pine among the six indigenous pine species of India, which is much valued for its timber and oleoresin. It occurs in the monsoon belt of the outer Himalaya, from North – eastern part of Pakistan to Arunachal Pradesh in India at elevations varying from 450 to 2300m asl. It is found distributed over a long strip of about 3,200 km between latitudes 26°N to36°N and longitudes 71°E to 93°E. The species is economically very important

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and is used for variety of purposes viz., timber for house construction, fuel wood extraction, charcoal formation, growing trees stems for resin tapping, needles for fuel briquetting, cattle bedding and manufacturing organic manure, etc.

Work on seed testing of various provenances of *Pinus roxburghii* from Uttarakhad and Himachal Himalaya has been done by Sharma et al. (Sharma et al., 2001), Ghildiyal et al. (2007), and Ghildiyal and Sharma (2005, 2007), whereas, Isoenzyme analysis on different provenances of Chir pine from the Indian subcontinent has been done by Hussain (1995) from Pakistan, and by Sharma (1999) and Sharma (2007) from India. The germination capacity of seeds is strongly influenced by abiotic factors such as temperature, water stress and, in certain cases, light, which often show a significant influence on germination (Knipe, 1973; Rao & Singh, 1985). Several growth hormones and chemicals like H_2O_2 have been found to enhance the rate of seed germination in may species viz., in spruce (Chandra & Chauhan, 1976), *Northofagus obliqua* and *N. procera* (Shafiq, 1980), *Pinus wallichiana* (Thapliyal et al., 1985), V, *Pinus roxburghii* (Ghildiyal, 2003) etc, through which losses in seed germination could be minimised (Quarberg & Jahns, 2000). In this study we have tried to assess the variation in seed characters and an effort was made to explore the efficacy of hydrogen peroxide treatment on the germination and seedling characters of 16 provenances of *Pinus roxburghii* collected from Garhwal Himalaya.

Materials and methods

The study was conducted on the seed and seedling characteristics of 16 provenances of *Pinus roxburghii* collected from different geographic locations, which were distributed in 4 districts i.e., Pauri, Chamoli, Rudraprayag and Tehri of Uttarakhand state in India (latitude 29° 26′ to 31° 28′ N and longitude 77° 49′ to 80° 06′E). The majority of rain fall (1000mm to 1800mm) in these regions occur during monsoon period i.e., from June to September, and are represented by sub-tropical to temperate climates. The detailed geographical and meteorological attributes of various provenances are given in *Table 1. & Fig.1.*

Table 1. *Geographical and meteorological descriptions of different provenances of Pinus roxburghii.*

Provenance	District	Latitude (N)	Longitude (E)	Altitude (m)	Temperature Min.	Temperature Max.	Mean annual rainfall (mm)
Ashtavakra	Pauri	30° 13’	78° 48’	960	5.76	37.70	705.00
Agustmuni	Rudraprayag	30° 23’	79^0 02’	875	4.31	36.59	833.00
Badiyargarh	Tehri	30° 17’	78^0 50’	1080	7.50	36.30	930.00
Ghansali	Tehri	30° 27’	78° 39’	890	5.00	34.60	1230.00
Godnar	Chamoli	30° 30’	79°16’	1680	1.30	24.00	1890.00
Jaiharikhal	Pauri	29° 47’	78° 32’	960	7.54	37.00	1150.00
Jasholi	Rudraprayag	30° 16’	79° 04’	1520	1.60	34.10	1025.00
Kalimath	Chamoli	30° 34’	79° 05’	1540	1.60	26.10	1257.50
Lansdowne	Pauri	29° 50’	78° 41’	1703	-0.90	25.80	1260.00
Mayali	Tehri	30° 23’	78° 47’	1400	2.60	25.10	1030.00
Pabo	Pauri	30° 15’	79° 01’	1640	1.8	32.4	875.00
Pauri	Pauri	30° 09’	78° 48’	1660	-0.48	26.30	1792.00
Pokhal	Tehri	30° 25’	78° 59’	820	5.70	37.63	800.00
Tangni	Chamoli	30° 29’	79° 28’	1480	4.20	25.50	990.00
Thalisain	Pauri	30° 02’	79° 03’	1640	1.9	31.00	1025.00
Vana	Chamoli	30° 38’	79° 05	1610	1.30	24.00	1660.00

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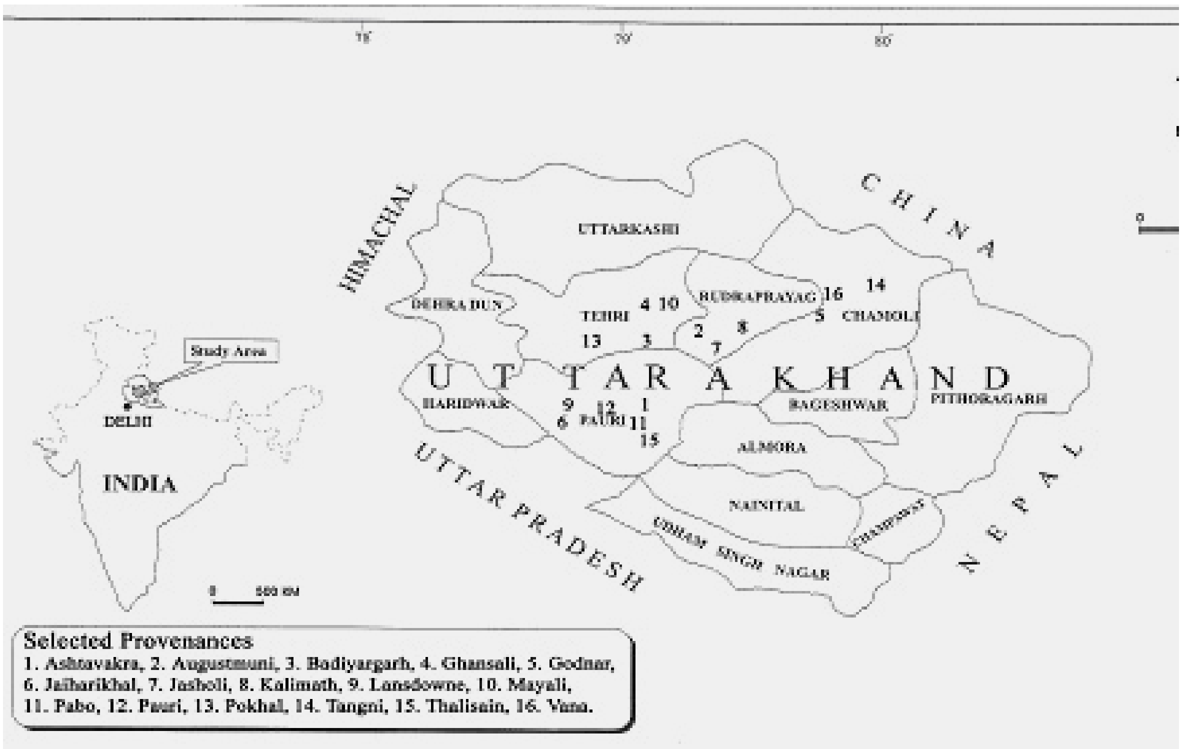

Figure 1. *Location map of the study areas*

The cones were collected from plus trees which were tallest, straightest, best shaped with well developed crowns and were free from pests and diseases. Ten ripe cones per tree were collected at random from the selected plus trees for recording the seeds length, width and thickness of randomly selected seeds (8 replicates of 20 seeds each) for each seed source. Seed weight of 800 seeds of each seed source (8 replicates of 100 seeds each) was recorded in order to verify the variation in seed weight. The seeds of different provenances having same level of ripeness were collected and subjected to viability test by floating method to select only the viable seeds. Seed germination in all the provenances was carried out under laboratory conditions at 25°C temperature, inside a seed germinator (Model No. 8LT-SGL CALTAN) after applying following presowing treatments to each set:

Treatment 1- Soaking of the seeds in distilled water at room temperature (25°C) for 24 hours.

Treatment 2- Soaking of the seeds in Hydrogen peroxide (H_2O_2 1% v/v) at room temperature (25°C) for 24 hours.

Observations were recorded daily regarding germinated /non-germinated seeds up to 21 days. Radical emergence was taken as the criteria for germinability. The collected data were further quantified in terms of percent germination and germination value. Percent germination was the value of seeds germinated at the completion of the germination period, whereas, germination value is an index, combining speed and completeness of germination; which according to Czabator (1962) can be expressed as: GV= PV X MDG, where, GV is germination value, PV is the peak value of germination, and MDG is the mean daily germination. The critical difference (CD) was calculated as: CD = SEd X $t_{0.01}$, Where, SEd is the standard error of difference calculated as SEd = $\sqrt{2Me/r}$, where Me= mean sum of square and r= number of

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replicates. The variation in growth and biomass production in different provenances was recorded by transferring the laboratory germinated seedlings to polythene bags containing potting mixture of sand, soil and FYM in the ratio of 1:1:1. The polythene bags containing seedlings were placed in randomized block design, with three replications of each provenance in the homogenous net house conditions. Initially, watering was done daily up to four weeks and thereafter, at 3 days interval, till the commencement of the rainy season. The data on height and root length were recorded at the end of four months.

Results and Discussion

The results obtained on seed characteristics are presented in *Table* 2.

Table 2. *Seed characteristics, Germination percentage and Seedling growth in different provenances of Pinus roxburghii from Garhwal Himalaya.*

Provenances	Seeds weight with wings (gm)	Seed Length (mm)	Seed Width (mm)	Seed Thickness (mm)
Pokhal	13.36 ±0.40	9.34 ±0.23	5.28 ±0.17	3.33 ±0.18
Godnar	11.02 ±0.53	8.25 ±0.20	4.72 ±0.28	2.82 ±0.14
Kalimath	14.00 ±0.51	9.42 ±0.33	5.35 ±0.21	3.61 ±0.21
Agustmuni	11.96 ±0.48	9.14 ±0.31	4.80 ±0.25	3.02 ±0.17
Jasholi	13.26 ±0.65	10.12 ±0.25	5.65 ±0.27	3.85 ±0.23
Tangni	15.02 ±0.55	10.80 ±0.27	5.80 ±0.21	4.23 ±0.20
Vana	13.76 ±0.59	10.65 ±0.21	5.84 ±0.27	4.12 ±0.18
Ashtavakra	12.66 ±0.38	10.08 ±0.27	5.35 ±0.22	3.86 ±0.15
Mayali	11.89 ±0.47	9.36 ±0.30	5.08 ±0.15	3.65 ±0.24
Jaiharikhal	12.78 ±0.60	9.40 ±0.35	5.56 ±0.23	3.23 ±0.20
Lansdowne	13.98 ±0.54	10.28 ±0.29	6.35 ±0.28	4.07 ±0.17
Pauri	14.27 ±0.42	9.99 ±0.20	5.51 ±0.21	3.49 ±0.19
Ghansali	14.88 ±0.49	10.56 ±0.29	5.89 ±0.26	4.01 ±0.14
Thalisain	15.67 ±0.37	10.98 ±0.24	6.98 ±0.18	4.40 ±0.21
Pabo	15.48 ±0.64	10.30 ±0.27	5.84 ±0.15	3.94 ±0.16
Badiyargarh	12.78 ±0.58	8.48 ±0.32	5.36 ±0.23	3.44 ±0.25
F-test	3.890*	0.0614	0.266	2.132*
P-value	0.0006	1.00	0.9956	0.0355
LSD	1.8174	0.8576	0.6126	0.5922
SD	0.803	0.559	0.447	1.339
CV%	8.176	10.006	12.117	9.881

*significant at 5% level.
**ArcSine values are given in parenthesis

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Table 2. cont.

Provenances	Germination percentage (control)**	Germination value (control)	Germination percentage (H_2O_2 1% v/v)**	Germination value ($H_2O_2$1% v/v)
Pokhal	82.2 ±2.06 (65.05)	10.88 ±1.18	88.4 ±0.75 (70.09)	18.29 ±1.61
Godnar	84.0 ±7.42 (66.42)	7.88 ±2.08	80.4 ±1.17 (63.72)	13.24 ±1.21
Kalimath	97.6 ±1.17 (81.09)	6.05 ±1.54	98.4 ±0.75 (82.73)	7.64 ±0.80
Agustmuni	75.2 ±1.02 (60.13)	18.39 ±2.57	83.2 ±1.02 (65.80)	14.97 ±0.94
Jasholi	73.2 ±1.86 (58.82)	6.35 ±0.79	80.0 ±1.42 (63.44)	6.10 ±0.81
Tangni	38.0 ±1.67 (38.06)	3.25±1.31	84.0 ±1.48 (66.42)	17.68 ±1.03
Vana	89.6 ±1.72 (71.19)	16.1±1.59	95.2 ±1.02 (77.34)	36.94 ±5.10
Ashtavakra	92.4 ±1.47 (74.00)	28.9±0.99	94.0 ±1.10 (75.82)	69.97 ±5.74
Mayali	81.6 ±1.17 (64.60)	24.43±1.99	88.0 ±0.90 (69.73)	66.62 ±2.87
Jaiharikhal	71.2 ±1.02 (57.54)	4.14 ±1.37	85.2 ±1.02 (67.37)	19.48 ±1.74
Lansdowne	79.6 ±1.60 (63.15)	3.61 ±0.73	92.4 ±0.75 (74.00)	25.64 ±1.59
Pauri	85.6 ±1.17 (67.70)	4.81 ±1.65	90.4 ±1.17 (71.95)	32.19 ±2.35
Ghansali	74.8 ±2.06 (59.87)	3.94 ±0.89	91.2 ±1.10 (72.74)	37.33 ±1.96
Thalisain	90.0 ±1.42 (71.56)	7.26 ±0.86	96.0 ±0.90 (78.46)	43.57 ±1.95
Pabo	84.0 ±2.42 (66.42)	5.49 ±0.94	85.2 ±1.02 (67.37)	24.38 ±1.69
Badiyargarh	39.2 ±1.36 (38.76)	2.84 ±0.74	83.6 ±1.33 (66.11)	17.85 ±2.33
F-test	48.6175*	45.049*	9.341*	212.436*
P-value	1E-17	3.15E-17	8.02E-08	1.17E-27
LSD	20.6650	10.2917	6.9224	23.4626
SD	16.773	8.036	5.702	18.896
CV%	21.674	83.321	6.445	66.905

*significant at 5% level.
**ArcSine values are given in parenthesis

Weight of 100 seeds in various provenances/seed sources ranged from 11.02 ±0.53g (Godnar) to 15.67 ±0.37g (Thalisain). The mean weight of 100 seeds in Pabo (15.48 ±0.64g), Tangni (15.02 ±0.55g), Ghansali (14.88 ±0.49g) and Pauri (14.27 ±0.42g) seed sources were close to the maximum seed weight. The maximum seed length was observed (10.98 ±0.24mm) for Thalisain seed source and minimum (8.25 ±0.20mm) for Godnar seed source. The existing variation for seed width was low as compared to seed length and thickness. The seed width varied between 4.72mm (Godnar) to 6.98mm (Thalisain), similarly, the maximum seed thickness (4.40 ±0.21mm) was revealed by Thalisain seed source, however, the thinnest seeds were produced by Godnar seed source (2.82 ±0.14mm).

Germination of seeds in various seed sources after pre-soaking treatment (H_2O_2 1%v/v) under 25°C temperature has yielded significant differences in seed germination. The maximum germination percentage (98.4 ±0.75%) was recorded for Kalimath seed source, and minimum (80.0 ±1.42%) for Jasholi seed source. However, the higher germination value (69.97 ±5.74) was recorded for Ashtavakra, and lower (6.10 ±0.81) for Jasholi seed source. Under control conditions the highest germination percentage (97.6 ±1.17%) was recorded for Kalimath seed source and lowest (38.0 ±1.67%) for Tangni seed source. Similarly, the highest germination value (28.09 ±0.99) was recorded for Ashtavakra, whereas, lowest germination value for Badiyargarh seed source (2.84 ±0.74).

The performance of seedlings raised from Pabo seed source was superior, having maximum height (10.16 ±0.37cm), and minimum seedling height (8.70 ±0.29cm) was recorded for Agustmuni seed source. The highest root length was recorded for the

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seedlings of Pabo seed source (14.62 ±0.53cm), and lowest (12.38 ±0.63cm) for Mayali seed source (*Table 2.*).

Simple correlation was calculated between seed characteristics, germination characteristics and geographic parameters viz., altitude, mean annual rainfall (mm), longitude and latitude, etc., results of which are presented in *Table 3*.

Table 3. *Simple correlation coefficients between seed characteristics and geographic variation.*

Characters	1	2	3	4	5	6
Seed length (mm)	1.000					
Seed width (mm)	0.797*	1.000				
Seed thickness (mm)	0.904*	0.840*	1.000			
100-Seed weight (gm)	0.811*	0.812*	0.783*	1.000		
Germination % (control)	0.124	0.048	-0.005	-0.007	1.000	
Germination value	-0.074	-0.379	-0.086	-0.479	0.394**	1.000
Germination % ($H_2O_2$1%v/v)	0.464**	0.470**	0.499**	0.429**	0.579**	0.189
Germination value	0.302**	0.152	0.354**	-0.007	0.314**	0.695*
Seedling height (cm)	0.290	0.228	0.410**	0.476**	-0.169	-0.280
Root length (cm)	-0.066	-0.015	0.016	0.264	-0.062	-0.487
Altitude (m)	0.198	0.331**	0.338**	0.108	0.103	0.119
Mean annual rainfall (mm)	-0.074	-0.135	-0.035	-0.122	-0.309	0.014
Latitude N	-0.182	0.031	-0.196	-0.219	0.215	0.046
Longitude E	0.247	0.550**	0.377**	0.247	0.290	-0.113

Significant at 1% level & ** significant at 5% level

Characters	7	8	9	10	11	12
Seed length (mm)						
Seed width (mm)						
Seed thickness (mm)						
100-Seed weight (gm)						
Germination % (control)						
Germination value						
Germination % ($H_2O_2$1%v/v)	1.000					
Germination value	0.447**	1.000				
Seedling height (cm)	-0.121	-0.174	1.000			
Root length (cm)	-0.129	-0.494	0.789*	1.000		
Altitude (m)	0.119	0.371**	0.185	0.016	1.000	
Mean annual rainfall (mm)	-0.340	-0.023	0.079	-0.020	0.556	1.000
Latitude N	0.076	-0.091	-0.289	-0.286	-0.047	0.093
Longitude E	0.199	0.103	0.202	0.049	0.396**	0.090

Significant at 1% level & ** significant at 5% level

Statistically negative correlation was observed between germination value and seed characteristics. A significant positive correlation was observed between seed germination (H_2O_2 1% v/v) and other seed parameters. Latitude and longitude were found to be significantly and positively correlated with germination of chirpine seeds under normal (control) conditions (0.215 and 0.290) and when treated with H_2O_2 (0.076 and 0.199). Germination value of the H_2O_2 treated seeds showed negative correlation

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with latitude, and positive correlation with longitude, whereas, seeds under controlled conditions showed positive correlation with latitude and negative correlation with longitude. Correlation between germination percentage and rainfall was negative in all the seeds treated and germination under controlled conditions, whereas significantly positive correlation was observed between rainfall and altitude (r = 0.556). Except for seedling height and germination value of seeds grown under controlled conditions all the other parameters of seed characteristics and germination characteristics were found to be negatively correlated with rainfall. H_2O_2 treatment showed significant effect on seed germination percent in all the seed sources except Godnar seed source (*Table 2*).

It is apparent from the results that seed sources, which had higher values for seed parameters, also showed better performance in germination. These findings have been supported by the concept of Baldwin (1942) and Dunlap and Barnett (1983), according to which, seed size and weight have pronounced effects on seed germination. Generally, large seeds have fast and uniform germination, due to more endosperm nutrient pool (Kandya, 1978). Therefore, seed source variation in germination percent and related traits may be ascribed to the differences in seed dimensions and weight. Germination values varied considerably among seed sources and exhibited a random pattern. Germination value is an index of combining speed and completeness of germination, which itself is a function of seed size and weight (Czabator, 1962; Dunlap & Barnett, 1983). On the other hand, variation observed in time taken to complete germination could be attributed to the differences in germination rate and germination value of the selected provenances. Significant variation in germination values among seed sources is in conformity with those found in fir and spruce by Singh and Singh (Singh & Virendra Singh, 1981).

Soaking the seeds for 24 hours in a solution of H_2O_2 (1% v/v) had a significant effect on the rate of germination and average germination percentage. About 88.5 % of mean average germination was revealed by the seeds that were treated with H_2O_2, while the mean average germination percentage of untreated (control) seeds was just 77.4 % (*Table 2*). Simultaneously, H_2O_2 treatment also caused an appreciable decrease, in shortening of germination period by 10 days. Similar results were recorded by Chandra and Chauhan (1976) in *Picea smithiana* and Shafiq (1980) in *Nothofagus obliqua* and *Nothofagus procera* seeds. Thus, soaking of chir-pine seeds in H_2O_2 (1% v/v) for 24 hours is highly beneficial and therefore for large scale germination, treatment of H_2O_2 should be preferred, since it also involves the saving of expenditure as compared to other hormones. The cost involved in case of H_2O_2 will be approximately half of the cost of other hormones. It was also evident from the results that although the H_2O_2 improved the germination of seeds in all the sources, still there were variations in its effect on rate of the germination in the provenances, which may be due to variation in geographical locations and the altitude of the seed sources (Holm, 1994; Barnett, 1997; Vera, 1997). Differences in the rate of germination in the provenances have also been documented by Webb and Farmer (1968), Wilcox (1968) and Tewari et al. (2001). It is clear from the results that the sexual reproductive efficiency, which can be assessed by determining germination capacity may vary with altitude. Thus, altitudinal provenances of a species may differ not only in seed germination but also in their reproductive efficiency.

The seed sources varied significantly among themselves with respect to field survival and growth parameters (*Table 2.*) during all stages of measurements. The seedlings of different provenances, when grown under common nursery environmental conditions,

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often displayed different patterns of shoot growth (Dormling, 1979; Rehfeldt & Wycoff, 1981). However, it is not always easy to show that such differences are adaptive, presumably in response to the environment of the parent seed origin. Analysis of variation in growth-related traits of several tree species indicated that some portion of the total variation might be under strong genetic control, which is of adaptive importance and leads to the differentiation into distinct populations. In the present investigation, since seeds of all seed sources were raised under common nursery conditions, the environmental influences being reduced to minimal, therefore, variation among seed sources in field emergence and growth may be interpreted as genetic. Sniezko and Stewart (1989) were of the view that the provenance, and within provenance variation in nursery traits is essentially genetic in nature.

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