IMPROVEMENT IN GERMINATION OF CHIRPINE (*PINUS ROXBURGHII*) BY A PRESOWING TREATMENT WITH HYDROGEN PEROXIDE

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Received July 2006

GHILDIYAL, S. K., SHARMA, C. M. & KHANDURI, V. P. 2007. Improvement in germination of chirpine (*Pinus roxburghii*) by a presowing treatment with hydrogen peroxide. Soaking of *Pinus roxburghii* seeds for 24 hours in H_2O_2 (1% v/v) solution showed 89.8% germination compared with 72.5% for seeds treated with distilled water (control) at 25 °C. H_2O_2 shortened the germination period by 10 days. Although the seeds of *P. roxburghii* germinate well due to lack of dormancy, the increasing demand for large quantities of seeds for reforestation programmes makes presowing treatments useful in improving the percentage of germination.

Keywords: Provenances, dormancy, temperature, correlation, geographic parameters

GHILDIYAL, S. K., SHARMA, C. M. & KHANDURI, V. P. 2007. Pembaikan percambahan *Pinus roxburghii* dengan rawatan prasemai menggunakan hidrogen peroksida. Biji benih yang direndam dalam larutan H_2O_2 (1% v/v) selama 24 jam pada 25 °C menunjukkan 89.8% percambahan berbanding dengan 72.5% dalam air suling. H_2O_2 memendekkan tempoh percambahan sebanyak 10 hari. Walaupun percambahan biji benih *P. roxburghii* baik kerana ia bukanlah dorman, permintaannya yang tinggi dalam program penghutanan semula menjadikan rawatan prasemai penting dalam memperbaiki kadar dan peratusan percambahan.

INTRODUCTION

Pinus roxburghii, commonly known as chirpine or Himalayan long needle pine, is a gregarious, fire resistant, indigenous tree species often forming pure forests between 1000 and 2300 m elevation in western-central Himalaya and constitute about one-third of the total forest area of Uttaranchal Himalaya (Tewari 1994). In nature, the forests of *P. roxburghii* are found approximately from longitudes 70° E to 93° E and latitudes 26° N to 36° N in a subtropical and warm temperate monsoon belt, between 450 and 2600 m in Siwaliks and Himalayan main river valleys, from Kashmir to Bhutan. Qualities such as a straight cylindrical bole, rapid growth, high volume returns and a capacity to colonize degraded habitats make this species a precious resource of Himalayan region. It has a great importance in Indian economy because it grows well on poor soils, under harsh climatic conditions, and yields timber and resin that have many uses. Due to its commercial importance, the chirpine is being planted on a massive scale in different parts of the country. The species is characterized by considerable natural variation like other wind-pollinated conifers. Therefore, the silvicultural and genetical aspects of this species need to be further investigated for improving out-planting techniques, field survival and genetic improvement of specific traits.

Selection of the best provenances of the desired species for a given site or region is necessary for achieving maximum productivity. The concept of provenance testing has now been well established and is in vogue in many developed countries of the world. In those species (for which there are data on individual tree variability), genetic differences are associated with place of origin, which have often been several times as great as those between individual trees in the same stand. Plus trees chosen for selective breeding, without

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regard to provenance performance may give birth to an inferior race. Therefore, it is particularly necessary to do provenance testing prior to more intensive breeding work. In some conifer species there are 4:1 differences in growth rate and correspondingly large differences in other traits between different races, and a breeder should be sure that he has the best race before starting crossing work and seed orchard establishment (Sharma *et al.* 2006).

Temperature affects the percentage and rate of germination (Landis *et al.* 1998) through its effects on seed deterioration, loss of dormancy (Wang & Berjak 2000) and the germination process itself (Roberts 1988, Bewley & Black 1994, Ghildiyal & Sharma 2005). At supra-optimal temperatures, germination often decreases (Totterdell & Roberts 1979, Murdoch *et al.* 1989). Some chemicals, such as hydrogen peroxide and other growth promoting substances have been found helpful in enhancing the rate of germination of seeds in many species (Chandra & Chauhan 1976, Shafiq 1980, Thapliyal *et al.* 1985, Ghildiyal 2003), through which losses in seed germination could be minimized (Quarberg & Jahns 2000). In the present study we explored the efficacy of hydrogen peroxide treatment on the germination of *P. roxburghii* seeds, taking into account various physiological processes in relation to post harvest desiccation.

MATERIALS AND METHODS

The present study was conducted in chirpine forests of two states viz. Uttaranchal (which is further divided into Garhwal and Kumaon Himalaya or central Himalaya) and Himachal Pradesh (Himachal Himalaya) situated in the western-central Himalayan region of India. The details of the study areas are presented in Table 1.

The studies pertaining to seed germination after pre-soaking treatment (at 25 °C) were carried out at various temperatures *viz.* 20, 25 and 30 °C using a regime of 12 hours light alternating with 12 hours dark inside a seed germinator. Seeds of all provenances of *P. roxburghii* were germinated at similar specified temperatures after applying the following 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.

The seeds of different provenances having the same level of ripeness were collected and subjected to viability test by the floating method. For germination, seeds in five replicates of 100 seeds each were placed in Petri dishes containing

Table 1 Geographic and climatic descriptions of the selected seed sources of *Pinus roxburghii*

Provenance	District /State	Latitude (N)	Longitude (E)	Altitude (m)	Temperature (°C)		Mean annual rainfall (mm)
					Min	Max	
Agustmuni	Rudraprayag (UA)	30° 23'	79° 02'	875	4.31	36.59	833
Ashtavakra	Pauri (UA)	30° 13'	78° 48'	960	5.76	37.70	705
Badiyargarh	Tehri (UA)	30° 17'	78° 50'	1080	7.50	36.30	930
Gallu	Mandi (HP)	31° 42'	77° 01'	1520	-0.20	31.40	1100
Ghansali	Tehri (UA)	30° 27'	78° 39'	890	5.00	34.60	1230
Jaiharikhal	Pauri (UA)	29° 47'	78° 32'	960	7.54	37.00	1150
Jasholi	Rudraprayag (UA)	30° 16'	79° 04'	1520	1.60	34.10	1025
Kaligad	Almora (UA)	29° 38'	79° 25'	1800	0.42	26.86	1060
Khamlekh	Pithoragarh (UA)	29° 57'	80° 04'	1450	3.10	31.20	1230
Lansdowne	Pauri (UA)	29° 50'	78° 41'	1703	-0.90	25.80	1260
Matiyal	Nainital (UA)	29° 10'	79° 20'	1740	3.80	23.40	2270
Matnoh	Hamirpur (HP)	31° 45'	76° 43'	980	0.80	33.60	1150
Nihari	Hamirpur (HP)	31° 29'	76° 28'	800	1.20	35.40	1125
Pabo	Pauri (UA)	30° 15'	79° 01'	1640	1.80	32.40	875
Patwadangar	Nainital (UA)	29° 16'	79° 20'	1500	7.40	28.50	2850
Ranital	Kangra (HP)	31° 10'	76° 05'	960	0.20	32.50	1350
Seshan	Shimla (HP)	31° 07'	77° 45'	1540	0.50	30.30	1075
Soni	Almora (UA)	29° 12'	79° 24'	1650	2.30	28.00	1040
Tangni	Chamoli (UA)	30° 29'	79° 28'	1480	4.20	25.50	990
Thalisain	Pauri (UA)	30° 02'	79° 03'	1640	1.90	31.00	1025

UA = Uttaranchal, HP = Himachal Pradesh

two filter papers, kept in the germinator and maintained at desired temperature. Observations were recorded daily regarding germinated /non-germinated seeds up to 21 days. Radicle emergence was taken as the criterion for germinability. The germinated seeds after the experiments were transplanted into polythene bags in the nursery.

The data on seed germination was recorded and quantified in terms of per cent germination and germination value. Per cent germination was the value of seeds that germinated at the completion of the germination period. Germination value is an index combining both speed and completeness of germination which according to Czabator (1962) can be expressed as GV= PV × MDG, where GV is germination value, PV is the peak value of germination, and MDG is mean daily germination.

The statistical analysis of each parameter was carried out on mean values and the analysis of variance (ANOVA) was performed using SPSS package. The critical difference (CD) was calculated as CD = SEd × $t_{0.01}$, where SEd is the standard error of difference calculated as SEd = √2Me/r, where Me is mean sum of square due to error and r = number of replicates.

RESULTS AND DISCUSSION

Seeds soaked in distilled water (control)

At 20 °C (Table 2) maximum germination percentage was recorded for Thalisain provenance (90.0%) and minimum for Tangni provenance (44.6%). However, maximum germination value (Table 3) was recorded for Agustmuni provenance (16.3) and minimum Ashtavakra provenance (0.8). On the other hand at 25 °C, maximum germination percentages were recorded for Ashtavakra (92.4%) and Thalisain (90.0%) provenances, and minimum for Tangni provenance (38.0%). However, higher germination value was recorded for Ashtavakra provenance (28.1), followed by Agustmuni provenance (18.4). Lower germination values were recorded for Khamlekh (2.3) and Matiyal (2.9) provenances. At 30 °C maximum germination percentage was recorded again for Thalisain provenance (80.0%), and the minimum for Kaligad provenance (20.6%). However, highest germination value was recorded for Pabo provenance (18.0) and lowest, for Kaligad provenance (0.2).

Table 2 Effect of hydrogen peroxide treatment on seed germination percentages (mean values ± standard error) of various provenances of *Pinus roxburghii* under different temperatures

	20 °C		25 °C		30 °C	
Provenance	Control	H_2O_2 (1%v/v)	Control	H_2O_2 (1%v/v)	Control	H_2O_2 (1%v/v)
Agustmuni	78.4 ± 2.62	88.2 ± 2.54	75.2 ± 1.02	83.2 ± 1.02	63.4 ± 1.08	93.6 ± 1.44
Ashtavakra	56.0 ± 6.78	92.0 ± 3.74	92.4 ± 1.47	94.0 ± 1.10	55.0 ± 1.73	86.0 ± 1.14
Badiyargarh	52.2 ± 1.58	78.2 ± 2.42	39.2 ± 1.36	83.6 ± 1.33	51.6 ± 1.21	64.2 ± 1.72
Gallu	75.6 ± 3.55	78.4 ± 3.34	73.2 ± 1.10	86.0 ± 0.71	65.7 ± 1.46	86.3 ± 1.66
Ghansali	72.4 ± 2.56	75.2 ± 1.21	74.8 ± 2.06	91.2 ± 1.10	75.4 ± 1.60	76.2 ± 1.27
Jaiharikhal	74.0 ± 2.76	77.0 ± 0.98	71.2 ± 1.02	85.2 ± 1.02	64.0 ± 1.58	75.8 ± 1.50
Jasholi	75.2 ± 1.94	84.0 ± 4.42	73.2 ± 1.86	80.0 ± 1.42	39.8 ± 1.56	57.6 ± 2.94
Kaligad	82.2 ± 1.26	90.6 ± 2 .38	80.4 ± 0.75	93.2 ± 1.10	20.6 ± 1.97	72.3 ± 1.67
Khamlekh	60.4 ± 1.55	84.2 ± 0.57	53.2 ± 1.10	85.8 ± 0.58	53.2 ± 1.14	79.6 ± 1.72
Lansdowne	82.6 ± 5.38	85.6 ± 2.72	79.6 ± 1.60	92.4 ± 0.75	75.3 ± 1.48	86.4 ± 1.36
Matiyal	64.2 ± 1.34	87.2 ± 0.92	60.0 ± 1.42	94.8 ± 1.10	65.5 ± 1.50	84.9 ± 1.71
Matnoh	84.0 ± 2.72	90.2 ± 1.48	80.0 ± 1.42	93.6 ± 1.17	53.4 ± 1.12	96.2 ± 1.53
Nihari	62.4 ± 4.12	80.6 ± 0.97	66.6 ± 0.93	93.6 ± 2.17	26.7 ± 0.93	46.6 ± 1.19
Pabo	86.4 ± 3.67	90.6 ± 4.35	84.0 ± 2.42	85.2 ± 1.02	75.2 ± 1.43	84.2 ± 2.20
Patwadangar	84.6 ± 2.14	94.0 ± 1.23	86.6 ± 0.51	98.0 ± 0.63	66.3 ± 1.01	86.7 ± 1.28
Ranital	68.2 ± 6.28	83.0 ± 2.72	73.2 ± 1.16	86.2 ± 0.86	40.9 ± 1.62	73.1 ± 1.79
Seshan	70.8 ± 2.39	78.0 ± 1.86	73.2 ± 1.10	92.2 ± 1.28	70.4 ± 1.44	83.8 ± 2.06
Soni	86.2 ± 0.86	97.4 ± 1.06	86.6 ± 0.93	98.4 ± 0.85	60.1 ± 1.46	71.7 ± 1.55
Tangni	44.6 ± 4.74	88.4 ± 2.86	38.0 ± 1.67	84.0 ± 1.48	73.0 ± 1.10	79.8 ± 1.72
Thalisain	90.0 ± 5.48	94.0 ± 4.60	90.0 ± 1.42	96.0 ± 0.90	80.0 ± 1.92	96.2 ± 0.86
Mean	72.5	85.8	72.5	89.8	58.8	79.1
CD at 1%	4.64	3.76	4.80	3.70	4.84	4.20

Table 3 Effect of hydrogen peroxide treatment on germination values (mean values ± SE) of various provenances of *Pinus roxburghii* under different temperatures

	20 °C		25 °C		30 °C	
Provenance	Control	H_2O_2 (1%v/v)	Control	H_2O_2 (1%v/v)	Control	H_2O_2 (1%v/v)
Agustmuni	16.32 ± 1.42	12.72 ± 0.83	18.39 ± 2.57	14.97 ± 0.94	7.09 ± 1.28	21.24 ± 0.97
Ashtavakra	0.82 ± 0.20	4.50 ± 1.06	28.09 ± 0.99	69.97 ± 5.74	3.70 ± 1.05	21.85 ± 1.23
Badiyargarh	4.62 ± 1.76	12.68 ± 1.42	2.84 ± 0.74	17.85 ± 2.33	1.59 ± 0.36	2.67 ± 0.55
Gallu	6.34 ± 0.92	9.47 ± 0.93	7.81 ± 0.86	7.02 ± 1.06	1.96 ± 0.37	4.44 ± 0.69
Ghansali	3.47 ± 0.67	13.47 ± 2.42	3.94 ± 0.89	37.33 ± 1.96	2.70 ± 0.83	9.31 ± 0.86
Jaiharikhal	6.81 ± 0.76	15.44 ± 2.08	4.14 ± 1.37	19.48 ± 1.74	4.51 ± 1.38	18.81 ± 1.09
Jasholi	4.54 ± 0.88	4.12 ± 0.74	6.35 ± 0.79	6.10 ± 0.81	1.70 ± 0.43	1.95 ± 0.42
Kaligad	5.14 ± 1.67	8.59 ± 1.44	5.09 ± 0.70	16.70 ± 1.17	0.22 ± 0.02	3.88 ± 0.87
Khamlekh	3.52 ± 1.52	9.42 ± 0.64	2.29 ± 0.88	6.19 ± 1.42	1.44 ± 0.24	4.80 ± 0.47
Lansdowne	4.52 ± 0.45	17.86 ± 1.37	3.61 ± 0.73	25.64 ± 1.59	3.54 ± 1.04	7.63 ± 0.94
Matiyal	2.29 ± 1.74	6.61 ± 1.24	2.85 ± 0.64	8.89 ± 1.42	6.35 ± 0.40	5.42 ± 0.57
Matnoh	8.27 ± 0.76	6.93 ± 0.70	6.29 ± 1.26	11.27 ± 0.61	0.72 ± 0.05	2.18 ± 0.50
Nihari	4.21 ± 1.42	5.68 ± 1.80	3.46 ± 0.96	9.12 ± 0.50	0.39 ± 0.04	0.90 ± 0.04
Pabo	7.25 ± 0.84	9.21 ± 2.67	5.49 ± 0.94	24.38 ± 1.69	17.98 ± 2.00	13.60 ± 0.93
Patwadangar	7.23 ± 0.48	11.75 ± 1.86	6.04 ± 1.69	17.44 ± 1.19	1.80 ± 0.41	5.26 ± 0.79
Ranital	3.88 ± 1.78	7.25 ± 2.07	5.75 ± 1.81	12.48 ± 0.68	0.33 ± 0.06	3.70 ± 0.86
Seshan	6.43 ± 0.79	7.85 ± 0.81	6.51 ± 1.36	9.36 ± 0.70	2.21 ± 0.45	4.86 ± 1.01
Soni	8.76 ± 0.93	14.37 ± 1.32	8.12 ± 1.16	17.06 ± 0.93	2.63 ± 0.54	4.62 ± 0.60
Tangni	5.38 ± 1.23	15.46 ± 1.16	3.25 ± 1.31	17.68 ± 1.03	6.16 ± 0.90	5.54 ± 0.47
Thalisain	2.74 ± 0.53	7.83 ± 1.31	7.26 ± 0.86	43.57 ± 1.95	3.33 ± 1.02	40.66 ± 1.51
Mean	5.63	10.06	6.88	19.62	3.52	9.17
CD at 1%	2.5	5.32	3.64	7.19	1.25	3.42

Soaking of seeds in H_2O_2 (1% v/v)

Some of the seed sources showed remarkable increase in germination percentage and germination value when their seeds were treated with H_2O_2 (1%v/v) as compared with those treated with distilled water (Tables 2 and 3). For example at 20 °C, Tangni provenance showed 44.6% seed germination and 5.4 germination value under normal (control) germination conditions, which was enhanced to 88.4% and 15.5 respectively, when the seeds were treated with H_2O_2. Similarly in Ashtavakra, Badiyargarh and Khamlekh provenances, which showed 56.0, 52.2 and 60.4% seed germination and 0.8, 4.6 and 3.5 germination values under normal (control) germination conditions, a considerable increase was noticed in germination percentages i.e. 92.0, 78.2 and 84.2%, and germination values, i.e. 4.5, 12.7 and 9.4 respectively after H_2O_2 treatment.

At 25 °C maximum increase in germination percentages, i.e. from (i) 38.0 to 84.0%, (ii) 39.2 to 83.6%, (iii) 66.6 to 93.6%, and (iv) 53.2 to 85.8% and germination values, i.e. from (i) 3.3 to 17.7, (ii) 2.8 to 17.9, (iii) 3.5 to 9.1,and (iv) 2.3 to 6.2 were recorded in (i) Tangni, (ii) Badiyargarh, (iii) Nihari, and (iv) Khamlekh seed sources respectively from normal (control) germination conditions to seeds that were treated with H_2O_2 (Tables 2 and 3).

At 30 °C, under normal (control) germination conditions (i) 63.4%, (ii) 55.0%, (iii) 53.4%, (iv) 20.6%, and (v) 66.3% germination percentages and (i) 7.1, (ii) 3.7, (iii) 0.7, (iv) 0.2, and (v) 1.8, germination values were recorded in (i) Agustmuni, (ii) Ashtavakra, (iii) Matnoh, (iv) Kaligad, and (v) Patwadangar provenances, which after H_2O_2 treatment were found to increase to (i) 93.6%, (ii) 86.0%, (iii) 96.2%, (iv) 72.3% and (v) 86.7%, and (i) 21.2, (ii) 21.9, (iii) 2.2, (iv) 3.9, and (v) 5.3 in these seed sources respectively (Tables 2 and 3).

Simple correlations were calculated between germination parameters and geographic factors (latitude, longitude, altitude and rainfall), and the results are presented in Table 4. Statistically significant negative correlations were observed between latitude and various parameters of germination percentage and germination value. Longitude was significantly positively correlated with germination of chirpine seeds treated with H_2O_2 at 20 °C (0.399) and normal (control) conditions at 30 °C (0.270). Germination values for all temperature treatments of H_2O_2-treated

Table 4 Correlation coefficients between germination parameters and geographic parameters of the seed sources of *Pinus roxburghii*

	Germination percentage						Germination value					
	20 °C		25 °C		30 °C		20 °C		25 °C		30 °C	
	Control	H_2O_2	Control	H_2O_2	Control	H_2O_2	Control	H_2O_2	Control	H_2O_2	Control	H_2O_2
Latitude	-0.174	-0.444	-0.087	-0.267	-0.155	-0.039	0.029	-0.351	0.060	-0.148	-0.203	-0.198
Longitude	-0.030	0.399	-0.092	0.018	0.270	0.147	-0.049	0.297	0.045	0.237	0.304	0.245
Altitude (m)	0.327	0.409	0.096	0.218	0.216	0.202	-0.149	0.066	-0.306	-0.173	0.204	-0.049
Rainfall (mm)	0.139	0.147	0.035	0.421	0.111	0.128	-0.103	0.025	-0.321	-0.255	-0.132	-0.245

seeds were positively correlated, whereas in normal (control) conditions only seeds at 30 °C showed positive correlation with longitude. Correlation between germination percentage and rainfall was positive in all the treated and control seeds. Positive correlation was observed between rainfall and seeds treated with H_2O_2 at 25 °C (r = 0.4210). Except for H_2O_2 treatment at 20 °C all the other parameters of germination value were found to be negatively correlated with rainfall.

Therefore, 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 germination percentage. About 89.8% germination was achieved with seeds that were treated with H_2O_2, while the mean germination percentage of untreated (control) seeds was just 72.5 % (Table 2). Simultaneously, H_2O_2 treatment also shortened the germination period by 10 days. Similar results were recorded by Chandra and Chauhan (1976) for *Picea smithiana* and Shafiq (1980) for *Nothofagus obliqua* and *N. procera* seeds.

Thus, soaking of chirpine 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 with other hormones. The cost involved in using H_2O_2 will be approximately half the cost of other hormones. It is also evident from the results that although H_2O_2 improved the germination of seeds of all the sources, still there were variations in its effect on rate of the germination in the provenances. This 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 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 practical implication of the study is that seeds of this commercially important tree species should be pre-treated with H_2O_2 (1% v/v) for 24 hours to optimize germination. However, it seems that the seed source is also as important as pre-treatments. Hence, seed collection should be carried out at appropriate locations because seed quality has definite relations to some selective range of sources. The present results have emanated from the *P. roxburghii* seedlots, which are considered important in forestry practices.

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