Effect of cold stratification on the germination of seeds of chirpine (*Pinus roxburghii* Sargent) from Indian Himalayan Region.

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Abstract: The seeds of *Pinus roxburghii* germinate well in nature, but due to increasing demand of large quantities of seeds for afforestation programmes, ways to accelerate the rate of germination are needed. Present study reports the beneficial effect of pre-chilling on the germination of seeds of 30 provenances of chirpine collected from the Uttarakhand and Himachal Pradesh states of India. The results are being discussed in terms of value of the practice for raising nursery seedling production. Cold stratification for 15 days improved the rate and percentage of germination of the non-dormant chirpine seeds, when germinated at 20°C and 25°C, whereas percentage of germination decreased in most of the provenances, germinated at 30°C. Germination of pre-chilled seeds of the Kalimath and Pokhal seed sources was found maximum (96% each), while minimum (62%) germination was observed for Chotti Singri seed source at 20°C. [Nature and Science. 2009;7(8):36-43]. (ISSN 1545-0740).

Key words: Germination percentage, Gibberellic acid, dormancy, temperature, afforestation.

1. Introduction

For enhancing the rate and percentage of germination and for breaking the dormancy, moist chilling or cold stratification has been widely used as a pre-sowing treatment (Schopmeyer, 1974; AOSA, 1992; ISTA, 1999; Wang and Berjak, 2000). This is simple, inexpensive and effective technique for overcoming the seed dormancy. Though the phenomenon is not yet fully known, but the effects of moist chilling in establishing hormonal levels have been proved due to initiation of appropriate enzyme activity (Nikolaeva, 1977).

Gibberellic acid (GA_3) has been shown to promote germination of seeds (Vogt, 1970; Krishnamurty, 1973; Chandra and Chauhan, 1976; Ghildiyal, 2003). The germination percentage increased in the seeds of *Nothofagus obliqua*, when pre-chilled after soaking in GA_3 solutions for 24 hours (Shafiq, 1980). Singh (1973) reported that spruce seeds germinate comparatively more profusely than silver fir, and every year enough seeds become available for raising sufficient planting stock. Parma Nand and wright (1922) have noticed that germination capacity of spruce seeds collected from trees of different diameter classes, have shown maximum 47% germination. Singh et al. (1975) have recorded 56% germination of spruce seeds in B.O.D. incubator and maximum 39.5% germination in pot culture experiments. On the other hand, pre-chilling or cold stratification has been found to be an effective method for germinating seeds of many other species (Shafiq and Omer, 1969; Miller, 1971; Stilinovic and Tucovic, 1971).

Work on seed testing of various provenances of *Pinus roxburghii* from Uttarakhand and Himachal Himalaya has been done by Sharma et al. (2001), Ghildiyal et al. (2007, 2008, 2009) and Ghildiyal and Sharma (2005, 2007). Studies on both physiologically dormant *Picea glauca* and non-dormant *Picea mariana* (black spruce) seeds have shown that moist chilling was beneficial in accelerating the rate of germination (Wang, 1987). Moreover, the phenomenon of cold stratification has long been recognized in overcoming physiological dormancy of seeds of many species. The work of Wang (1973 and 1987) on *Pinus* species and *Picea* species indicates that short periods of moist chilling of such non-dormant seeds may generally be efficacious, particularly if damage has accumulated due to natural deterioration or as a result of an imposed accelerated ageing regime. The hypothesis, therefore, is that the repair mechanism may be activated during moist chilling on non-dormant, cold temperate gymnospermous seeds, which are naturally aged or have been subjected to accelerated ageing. If this is generally applicable, cold stratification could have far-reaching implications for nursery practice, at least for such gymnosperms, in which aged, stored seed lots are used. In the present study we have tried to explore the effect of cold stratification on seeds of *Pinus roxburghii*, taking into account various physiological processes in relation to post harvest desiccation and chilling requirements.

2. Materials and Methods

The seeds were collected from the natural chirpine forests of two states viz., Uttarakhand (which is further divisible into Garhwal and Kumaon Himalaya or Central Himalaya) and Himachal Pradesh (Himachal Himalaya), situated in the Western-central Himalayan region of India. In Garhwal region the seeds of *Pinus roxburghii* were collected from the natural forests of four districts viz.; Pauri, Tehri, Rudraprayag and Chamoli (between 29° 20' to 31° 5' 30" N latitudes and 78° 15' to 80° 8' E longitudes). Whereas in Kumaon Himalaya from three districts viz., Nainital, Pithoragarh and Almora (the region extends between 28° 43' 24" N and 31° 27' 50" N latitudes and between 77° 34' 27" E and 81° 02' 22" E longitudes). In Himachal Himalaya the seeds were collected from five districts viz., Mandi, Solan, Hamirpur, Kangra and Shimla, situated between 30° 22' N to 33° 13' N latitudes and 75° 23' 24" E to 79° 00' 50" E longitudes. The details of the study areas have been presented in Table 1 and Figure 1.

Table 1. Geographic and climatic details 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.	
Ashtavakra	Pauri (U.K.)	30° 13'	78° 48'	960	5.76	37.70	705.0
Agustmuni	Rudraprayag (U.K.)	30° 23'	79° 02'	875	4.31	36.59	833.0
Badiyargarh	Tehri (U.K.)	30° 17'	78° 50'	1080	7.50	36.30	930.0
Chhoti Singri	Mandi (H. P.)	31° 49'	76° 59'	1220	0.30	32.50	1025.0
Dhulcheena	Almora (U.K.)	29° 42'	79° 49'	1850	-0.14	26.40	1125.0
Gallu	Mandi (H. P.)	31° 42'	77° 01'	1520	-0.20	31.40	1100.0
Ghansali	Tehri (U.K.)	30° 27'	78° 39'	890	5.00	34.60	1230.0
Godnar	Chamoli (U.K.)	30° 30'	79°16'	1680	1.30	24.00	1890.0
Jasholi	Rudraprayag (U.K.)	30° 16'	79° 04'	1520	1.60	34.10	1025.0
Jaiharikhal	Pauri (U.K.)	29° 47'	78° 32'	960	7.54	37.00	1150.0
Kaligad	Almora (U.K.)	29° 38'	79° 25'	1800	0.42	26.86	1060.0
Kalimath	Chamoli (U.K.)	30° 34'	79° 05'	1540	1.60	26.10	1257.5
Khamlekh	Pithoragarh (U.K.)	29° 47'	80° 04'	1450	3.10	31.20	1230.0
Lansdowne	Pauri (U.K.)	29° 50'	78° 41'	1703	-0.90	25.80	1260.0
Matiyal	Nainital (U.K.)	29° 10'	79° 20'	1740	3.80	23.40	2270.0
Matnoh	Hamirpur (H. P.)	31° 45'	76° 43'	980	0.80	33.6	1150.0
Mayali	Tehri (U.K.)	30° 23'	78° 47'	1400	2.60	25.10	1030.0
Nagali	Solan (H. P.)	30° 54'	77° 12'	1545	0.50	34.1	1000.0
Nihari	Hamirpur (H. P.)	31° 29'	76° 28'	800	1.20	35.4	1125.0
Pabo	Pauri (U.K.)	30° 15'	79° 01'	1640	1.8	32.4	875.0
Patwadangar	Nainital (U.K.)	29° 16'	79° 20'	1500	7.40	28.50	2850.0
Pauri	Pauri (U.K.)	30° 09'	78° 48'	1660	-0.48	26.30	1792.0
Pokhal	Tehri (U.K.)	30° 25'	78° 59'	820	5.70	37.63	800.0
Ranital	Kangra (H. P.)	31° 10'	76° 05'	960	0.20	32.5	1350.0
Seshan	Shimla (H. P.)	31° 07'	77° 45'	1540	0.50	30.3	1075.0
Seuri	Mandi (H. P.)	31° 50'	77° 02'	1460	0.15	33.2	925.0
Soni	Almora (U.K.)	29° 12'	79° 24'	1650	2.3	28.00	1040.0
Tangni	Chamoli (U.K.)	30° 29'	79° 28'	1480	4.20	25.50	990.0
Thalisain	Pauri (U.K.)	30° 02'	79° 03'	1640	1.9	31.00	1025.0
Vana	Chamoli (U.K.)	30° 38'	79° 05'	1610	1.30	24.00	1660.0

U.K.= Uttarakhand, H.P.= Himachal Pradesh

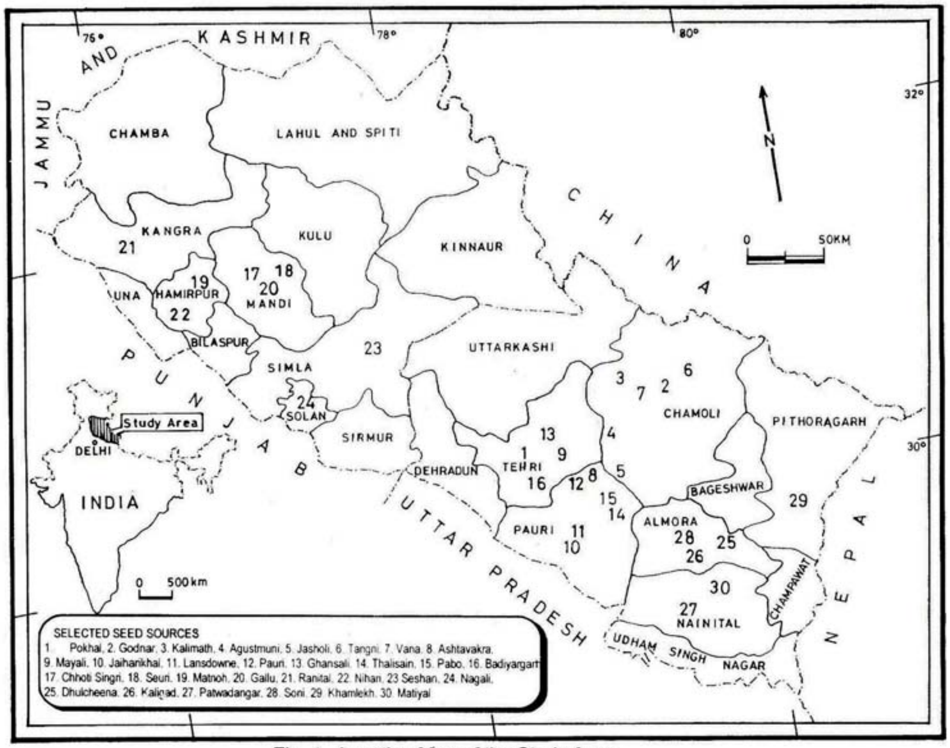

Fig. 1. Location Map of the Study Area

The germination tests on all the 30 seed sources were carried out under laboratory conditions at various temperatures *viz*., 20°C, 25°C and 30°C using regime of 12h light alternating with 12h dark inside a seed germinator (Model No. 8LT-SGL CALTAN). In each temperature regime, the seeds of all the seed sources were tested for germination with the following treatments:

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

Treatment 2- Seeds treated with Gibberellic acid (GA_3 10 mg l^{-1}) as above at room temperature (25°C) for 24 hour and then chilled for 15 days (at 3°C).

Chilling of non-dormant seeds was carried out in folded-over-polythene bags at 3°C for a period of 15 days (as per method suggested by Tompsett and Pritchard, 1998), after soaking the seeds for 24 hours in 10 mgl^{-1} Gibberellic acid, followed by subsequent drying. The chilled seeds were then subjected to various temperature treatments. Seed germination tests were carried out under similar conditions as described above. The progress of germination was monitored daily, until there was no further germination for a few days.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. For germination, the seeds in five replicates of 100 seeds each were placed in Petri dishes (diameter- 10 cm) containing two filter papers, kept in the germinator, and maintained at desired temperature. Observations were recorded daily 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 both 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 statistical analysis of each parameter was carried out on mean values and the analysis of variance (ANOVA) was performed using SPSS package (version 12.0). 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 replicates.

3. Results

Germination of seeds of various provenances after pre-chilling treatment under different temperature regimes, (i.e., 20°C, 25°C and 30°C) has yielded significant differences. The data analysed for its variance, has revealed prominent differences amongst different seed sources, which have been presented in Table 2 & 3. The detailed treatment-temperature interactions are given below:-

Table 2. Mean germination percentage of pre-chilled and un-chilled seeds in various provenances of *Pinus roxburghii* at different temperatures (Mean ± SE).

Provenance	Pre-chilled 20°C	Pre-chilled 25°C	Pre-chilled 30°C	*F-value*	P-value	Un-chilled 20°C	Un-chilled 25°C	Un-chilled 30°C	*F-value*	*P-value*
Agustmuni	86.2 ±4.94	84.5 ±0.91	79.2 ±4.64	0.25	0.79	85.2 ±2.10	81.2 ±1.10	87.8 ±1.50	1.29	0.34
Ashtavakra	75.0 ±8.95	92.0 ±1.28	56.4 ±1.92	4.14	0.07	70.0 ±8.95	89.6 ±1.17	54.0 ±1.52	8.43*	0.02
Badiyargarh	73.4 ±2.19	67.0 ±1.25	72.4 ±4.23	0.09	0.91	69.6 ±5.62	61.2 ±1.10	81.0 ±1.18	0.23	0.80
Chhoti Singri	62.0 ±4.26	78.4 ±0.86	51.3 ±2.56	1.12	0.39	56.2 ±3.92	73.2 ±1.20	48.1 ±1.65	0.72	0.52
Dhulcheena	94.6 ±2.56	98.8 ±0.92	63.2 ±3.24	1.43	0.31	92.6 ±1.78	93.4 ±1.75	65.5 ±1.31	1.48	0.30
Gallu	78.6 ±8.23	70.4 ±1.48	68.4 ±2.83	0.20	0.82	72.6 ±6.65	66.0 ±1.30	66.9 ±1.41	1.00	0.42
Ghansali	68.5 ±4.36	66.4 ±1.18	62.6 ±3.54	0.44	0.66	63.6 ±4.35	63.2 ±1.10	70.0 ±1.70	0.25	0.79
Godnar	92.4 ±5.62	93.2 ±1.86	82.4 ±2.86	2.25	0.19	90.2 ±1.12	90.2 ±1.10	89.6 ±1.63	0.08	0.93
Jaiharikhal	70.8 ±3.87	68.5 ±0.75	61.8 ±4.27	1.09	0.39	68.4 ±3.63	62.8 ±1.20	59.8 ±1.72	0.96	0.43
Jasholi	72.5 ±7.26	70.8 ±1.19	46.5 ±6.12	16.91**	0.00	69.6 ±4.72	65.6 ±1.72	47.6 ±1.21	2.07	0.21
Kaligad	90.2 ±3.68	94.5 ±1.36	75.6 ±2.48	3.04	0.12	88.2 ±3.25	90.2 ±1.60	73.1 ±1.85	1.81	0.24
Kalimath	96.0 ±8.00	88.0 ±1.52	88.0 ±8.92	0.17	0.85	94.0 ±4.00	82.0 ±1.42	86.6 ±1.81	0.39	0.69
Khamlekh	83.2 ±4.63	66.0 ±1.24	58.2 ±2.52	0.53	0.61	75.4 ±0.85	60.8 ±1.86	56.1 ±1.32	2.20	0.19
Lansdowne	82.0 ±5.59	76.3 ±1.42	74.5 ±2.75	0.48	0.64	75.8 ±6.12	73.6 ±1.17	80.6 ±1.29	0.17	0.85
Matiyal	87.5 ±2.27	86.2 ±1.39	88.6 ±1.82	0.01	1.00	83.2 ±1.93	83.8 ±1.36	97.2 ±0.86	0.03	0.97
Matnoh	90.3 ±5.45	94.0 ±0.96	72.0 ±3.16	1.23	0.36	87.4 ±2.74	90.6 ±0.75	79.4 ±2.02	3.21	0.11
Mayali	77.2 ±6.22	84.2 ±0.96	60.2 ±2.63	65.44**	0.00	72.2 ±5.27	79.6 ±1.17	64.2 ±1.99	1.62	0.27
Nagali	81.6 ±6.23	97.8 ±1.56	81.3 ±1.76	1.36	0.33	79.4 ±5.56	94.6 ±1.47	85.8 ±1.56	0.44	0.66
Nihari	77.5 ±6.46	88.0 ±1.72	52.8 ±2.94	8.47*	0.02	74.8 ±1.21	85.4 ±1.66	49.9 ±1.51	2.63	0.15
Pabo	76.2 ±6.66	69.2 ±1.62	84.2 ±2.68	0.35	0.72	72.4 ±7.28	65.2 ±1.50	82.4 ±1.36	0.01	0.99
Patwadangar	75.0 ±2.38	72.2 ±1.68	78.0 ±4.15	0.55	0.61	70.0 ±2.82	66.0 ±1.82	81.0 ±1.73	0.17	0.85
Pauri	88.6 ±4.73	90.6 ±1.26	44.0 ±5.09	21.68**	0.00	82.6 ±2.16	88.0 ±1.62	58.0 ±1.64	2.63	0.15
Pokhal	96.0 ±7.48	71.4 ±2.14	86.0 ±5.10	8.69*	0.02	94.0 ±2.45	65.2 ±1.40	94.0 ±1.23	6.77*	0.03
Ranital	80.0 ±2.17	75.8 ±1.19	75.6 ±4.28	1.32	0.33	75.0 ±4.32	73.6 ±0.75	73.9 ±1.50	2.22	0.19
Seshan	82.2 ±4.92	96.0 ±1.64	78.6 ±3.48	1.28	0.35	80.2 ±3.84	92.8 ±1.86	82.2 ±1.72	1.38	0.32
Seuri	84.8 ±7.67	86.2 ±1.12	80.7 ±1.84	0.02	0.98	80.0 ±8.39	83.8 ±0.68	86.4 ±0.82	1.78	0.25
Soni	90.8 ±6.59	88.4 ±1.45	80.4 ±2.91	7.22*	0.03	86.6 ±4.46	86.2 ±1.69	85.2 ±1.37	0.94	0.44
Tangni	70.3 ±5.75	67.2 ±1.73	68.3 ±4.36	0.24	0.79	66.0 ±2.42	62.0 ±1.42	66.8 ±1.72	0.71	0.53
Thalisain	88.0 ±5.83	87.6 ±1.21	78.0 ±6.63	0.91	0.45	86.0 ±6.00	83.2 ±1.10	89.4 ±1.36	0.57	0.60
Vana	94.0 ±2.44	92.1 ±0.89	80.0 ±2.84	23.48**	0.01	90.0 ±1.56	89.0 ±1.42	84.0 ±1.58	0.98	0.43
F- value	3.780**	2.505**	3.658**			4.182**	1.848**	3.202**		
P-value	6.75E-06	0.001594	1.51E-05			1.43E-06	0.022626	7E-05		
Mean	76.4	81.2	62.7			83.4	81.4	72.8		
Range	62.0-96.0	66.0-98.8	44.0-88.6			63.6-94.0	43.81-98.4	37.6-97.2		
C.D. at 1%	7.42	7.82	6.76			7.24	7.2	8.6		

***significant at 5% and *significant at 1% level*

Table 3. Germination value of pre-chilled and un-chilled seeds in various provenances of *Pinus roxburghii* at different temperatures.

Provenance	Pre-chilled			*F-value*	*P-value*	Un-chilled			*F- value*	*P-value*
	20°C	25°C	30°C			20°C	25°C	30°C		
Agustmuni	8.38 ±1.12	8.76 ±1.70	10.28±0.92	0.06	0.94	12.16±0.48	14.08±1.41	14.79±0.97	0.78	0.50
Ashtavakra	1.10 ±0.28	12.10 ±2.89	4.18 ±0.62	2.95	0.13	2.22 ±0.56	49.61±2.90	2.60 ±0.67	31.02**	0.00
Badiyargarh	2.27 ±0.68	5.37 ±1.43	6.25 ±0.84	0.61	0.57	2.18 ±0.32	6.38 ±1.13	4.47 ±0.96	1.34	0.33
Chhoti Singri	1.67 ±2.42	5.77 ±1.39	2.14 ±0.36	0.52	0.62	1.94 ±1.54	3.72 ±1.57	0.48 ±0.04	3.09	0.12
Dhulcheena	9.32 ±1.84	9.75 ±1.87	2.06 ±0.54	2.20	0.19	11.39±1.73	11.14±1.41	1.70 ±0.38	5.94*	0.04
Gallu	8.16 ±1.25	3.72 ±0.75	4.64 ±1.37	4.48	0.06	7.25 ±0.89	6.19 ±0.66	2.66 ±0.47	44.51**	0.00
Ghansali	3.88 ±1.61	7.86 ±1.66	5.35 ±0.42	0.81	0.49	3.52 ±0.59	5.40 ±1.24	7.27 ±0.86	1.51	0.29
Godnar	4.03 ±0.88	9.42 ±0.94	9.36 ±1.26	0.16	0.85	5.42 ±1.70	7.49 ±1.41	8.15 ±0.72	0.15	0.86
Jaiharikhal	6.16 ±0.91	6.48 ±1.54	8.74 ±0.86	0.02	0.98	8.62 ±0.81	8.34 ±1.42	10.38±0.98	0.29	0.76
Jasholi	2.71 ±0.96	5.28 ±1.36	3.42 ±0.56	13.72**	0.01	2.76 ±1.32	4.92 ±1.30	1.52 ±0.33	18.78**	0.00
Kaligad	6.44 ±1.12	4.63 ±0.74	2.35 ±1.07	1.96	0.22	6.67 ±0.96	5.98 ±0.98	1.83 ±0.34	33.94**	0.00
Kalimath	2.46 ±0.64	6.26 ±1.15	6.14 ±0.68	2.28	0.18	3.06 ±0.50	5.24 ±1.28	4.60 ±0.57	62.00**	0.00
Khamlekh	4.39 ±0.67	2.47 ±0.66	1.92 ±0.74	1.21	0.36	4.23 ±0.36	1.80 ±0.67	1.67 ±0.32	56.56**	0.00
Lansdowne	4.32 ±1.74	6.72 ±1.33	8.92 ±1.42	0.30	0.75	6.36 ±1.23	5.53 ±1.35	11.98±1.33	1.77	0.25
Matiyal	6.78 ±1.19	6.54 ±1.72	5.68 ±1.28	0.11	0.90	3.07 ±1.55	5.25 ±1.45	9.16 ±0.96	14.58**	0.00
Matnoh	5.63 ±0.69	6.48 ±1.36	2.26 ±0.54	9.76*	0.01	4.19 ±0.52	5.38 ±1.49	1.74 ±0.08	8.65*	0.02
Mayali	3.78 ±0.56	11.36 ±1.66	7.46 ±1.32	1.87	0.23	16.27±0.36	43.81±1.42	4.64 ±0.82	18.20**	0.00
Nagali	7.13 ±0.98	10.62 ±1.46	6.74 ±1.28	1.40	0.32	9.36 ±2.19	10.95±1.29	8.81 ±1.22	0.59	0.58
Nihari	6.86 ±1.54	6.23 ±1.17	1.68 ±0.39	7.28*	0.02	8.71 ±1.46	5.14 ±1.23	0.28 ±0.03	14.15**	0.01
Pabo	5.52 ±1.28	3.25 ±0.88	11.08±1.72	0.71	0.53	5.92 ±1.42	7.21 ±1.54	13.93±1.09	32.86**	0.00
Patwadangar	5.98 ±0.89	4.39 ±1.19	2.60 ±0.92	1.52	0.29	4.24 ±1.64	3.36 ±1.07	1.95 ±0.38	4.92	0.05
Pauri	7.25 ±1.33	10.42 ±1.21	4.56 ±0.78	0.94	0.44	10.71±0.77	17.46±1.17	2.30 ±0.56	4.75	0.06
Pokhal	2.00 ±0.54	4.34 ±0.76	5.21 ±0.82	1.23	0.36	3.43 ± 0.32	5.29 ±1.36	7.18 ±0.73	3.17	0.11
Ranital	4.62 ±1.77	2.08 ±1.26	1.52 ±0.42	1.69	0.26	2.63 ±1.21	4.03 ±1.33	0.25 ±0.02	28.64**	0.00
Seshan	5.92 ±1.27	8.76 ±0.98	4.25 ±0.64	8.86*	0.02	5.54 ±1.70	9.32 ±1.33	6.35 ±0.55	3.29	0.11
Seuri	4.34 ±0.94	6.32 ±0.84	1.94 ±0.33	7.27*	0.02	6.26 ±0.79	5.24 ±0.86	1.85 ±0.07	156.0**	0.00
Soni	8.75 ±1.36	10.08 ±2.03	3.21 ±1.15	5.38*	0.05	12.56±1.48	12.36±2.15	5.05 ±1.85	11.14**	0.01
Tangni	7.42 ±0.85	7.42 ±1.23	6.78 ±2.46	0.38	0.70	4.50 ±1.04	6.32 ±1.15	5.38 ±0.56	0.45	0.66
Thalisain	2.48 ±0.40	6.29 ±1.75	4.37 ±0.69	0.60	0.58	3.79 ±0.60	6.10 ±1.55	5.32 ±0.89	9.91*	0.01
Vana	5.25 ±1.48	7.94 ±0.92	4.53 ±1.21	1.36	0.32	13.64±0.43	15.86±0.95	6.26 ±0.67	66.53**	0.00
F- value	1.734**	1.446*	1.682**			21.325**	17.789**	10.348**		
P-value	0.036362	0.11393	0.045131			4.89E-22	5.33E-20	2.74E-14		
Mean	5.6	6.2	4.7			6.7	5.2	4.6		
Range	1.10-9.32	2.08-12.10	1.52-11.08			1.94-16.27	1.80-43.81	0.25-14.79		
C.D. at 1%	1.4	1.2	0.94			0.76	1.2	1.8		

***significant at 5% and *significant at 1% level*

Pre-chilling effects on germination

The presowing treatment of seeds by cold stratification enhances the rate and percentage of germination of seeds in many tree species. The results on the germination of pre-chilled and un-chilled seeds of 30 provenances of chirpine by subjecting some of the seeds to a chilling temperature of 3°C for 15 days and others without pre-chilling, after soaking with GA_3 and subsequently germinated at different constant temperatures i.e., 20°C, 25°C and 30°C have been presented in Table 2. The data reveals that in the pre-chilled seeds at 20°C, maximum germination (96.0 ±8.00%) was recorded in Kalimath seed source, whereas, minimum germination (62.0 ±4.26%) was recorded in the Chhoti Singri seed source. However, with a great similarity at 20°C the maximum and minimum germination percentages for un-chilled seeds were also recorded in Kalimath (94.0 ±4.00%) and Chhoti Singri (56.2 ±3.92%) seed sources, respectively. On the other hand, at 20°C the maximum germination value for pre-chilled seeds was recorded in Dhulcheena seeds source (9.32 ±1.84), and minimum in Ashtavakra seed source (1.10 ±0.28). However, at this temperature the maximum and minimum germination values for un-chilled seeds were recorded in Mayali (16.27 ±0.36) and Chhoti Singri (1.94 ±1.54) provenances, respectively (Table 3).

The pre-chilling of seeds followed by a temperature treatment of 25°C have shown highest germination percentage (98.8 ±0.92%) in Dhulcheena seed source, closely followed by several other seed sources, *viz*., Nagali (97.8 ±1.56%), Seshan (96.0 ±1.64%), Kaligad (94.5 ±1.36%) and Matnoh (94.0 ±0.96%). At 25°c the lowest germination percentage (66.0 ±1.24%) was recorded in Khamlekh seed source. Similarly for un-chilled seeds, maximum and minimum germination percentages were again recorded in Dhulcheena (95.4 ±1.75%) and Khamlekh (60.8 ±1.86%) seed sources, respectively. For pre-chilled seeds the highest germination value was recorded in Ashtavakra seed source (12.10 ±2.89), and lowest in Ranital seed source (2.08 ±1.26). On the other hand the highest and the lowest germination values for un-chilled seeds were recorded in Ashtavakra (49.61 ±2.90) and Khamlekh (1.80 ±0.67) provenances respectively.

The results obtained for pre-chilled and subsequent germinated seeds at 30°C (Table 2) ranged from 44.0 ±5.09% (Pauri) to 88.6 ±1.82% (Matiyal). The Kalimath (88.0 ±8.92%), Pokhal (86.0 ±5.10%), Pabo (84.2 ±2.68%) and Godnar (82.4 ±2.86%) seed sources were close to the maximum value. Minimum (44.0 ±5.09%) germination was recorded in Pauri seed source which was comparable to Jasholi (46.5 ±6.12%), Chhoti Singri (51.3 ±2.56%) and Nihari (52.8 ±2.94%) seed sources. A perusal of Table. 3 revealed that differences in germination values were wide-ranging i.e., 1.52 ±0.42 (Ranital) to 11.08 ±1.72 (Pabo) amongst the seed sources. It is clear from the results (Table 2) that a number of seed sources, particularly Agustmuni, Godnar, Lansdowne and Jaiharikhal, excelled over other seed sources. On the other hand, germination percentage and germination value in the un-chilled seeds of various provenances ranged from 47.6 ±1.21% (Jasholi) to 97.2 ±0.86% (Matiyal), and 0.25 ±0.02 (Ranital) to 14.79 ±0.97 (Agustmuni) respectively.

The analysis of variance (ANOVA) calculated between seed source and temperature for the germination of pre-chilled seeds has shown highly significant relationship at 5% level for some seed sources i.e., Jasholi, Mayali, Pauri and Vana, whereas, some other sources (Nihari, Pokhal and Soni) have shown significant relationship at 1% level. On the other hand, for the germination of un-chilled seeds, significant (at 1% level) relationship was obtained for Ashtavakera and Pokhal seed sources. The germination of the pre-chilled and un-chilled seeds of all the provenances has reflected highly significant relationship at 5% level.

4. Discussion

Cold stratification or chilling under moist conditions has long been recognised as a useful method of treating seeds to improve the rate and the percentage of germinability (Outcall, 1991). Heydecker and Coolbear (1977), suggested many other presowing treatments that increased germination percentage and rate. Thapliyal (1986) found that under favourable conditions, the seeds of *Pinus roxburghii* germinate well (60-80%) within 7-21 days. Because most of the seeds of chirpine are non-dormant, therefore it is often assumed that pre conditioning treatments are unnecessary in this species. However, our results have shown that pre-chilling treatment enhances rate of germination significantly at 20°C and 25°C. Our results are in conformation with Barnett (1971) who found that soaking of seeds in aerated water, promotes the germination of non-dormant seeds of South Pine. There are significant differences in germination between 15 days pre-chilled and un-chilled seeds of chirpine at 20°C and 25°C. Similar results were reported by Wang and Berjak (2000) between 14 days pre-chilled and un-chilled seeds of *Picea mariana*, which were subjected to different constant temperatures. Jones and Gosling (1994) and Jinks and Jones (1996) also reported that for the shallowly-dormant seeds of Douglas fir (*Pseudotsuga menziesii*), Lodgepole pine (*Pinus contorta*) and Sitka spruce (*Picea sitchensis*), moist-chilling is a requirement to alleviate dormancy. The beneficial effects of germination speed or rate (as a result of cold stratification), on quality and quantity nursery-grown seedlings were reported by Venator (1973) in Caribbean pine (*Pinus caribaea*), Mexal (1980) and Barnett and McLemore (1984) in Loblolly pine (*Pinus taeda*), and Logan and Pollard (1979) in Japanese larch (*Larix kaempferi*).

Although, the moist-chilling for 15 days did improve the rate and percentage of germination of the non-dormant chirpine seeds at 20°C and 25°C for over 21 days, but contradictory results were observed for seeds germinated at 30°C temperature. Pre-chilling treatment reduced germination percentage in most of the provenances at 30°C, whereas in remaining provenances there was not much difference in germination percentage between pre-chilled and un-chilled seeds at 30°C.

The effect of moist-chilling on the activation of germination in the chirpine seeds has not been previously reported and, therefore, is a new facet in understanding the benefits of short-term maintenance of seeds in a moistened condition at 3°C. The present results substantiate the beneficial effects of cold stratification on the release of dormancy and enhancing the rate of germination of chirpine seeds, which can be used to raise nursery for large scale afforestation programmes.

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