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GERMINATION AND BIOCHEMICAL CHANGES IN THE SEEDS OF CHILGOZA PINE (*PINUS GERARDIANA* WALL.) BY STRATIFICATION: AN ENDANGERED CONIFER SPECIES OF NORTH-WEST HIMALAYA

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SUMMARY

Natural regeneration is lacking in *Pinus gerardiana* Wall. commonly known as chilgoza and artificial regeneration has to be resorted to produce quality seedling for planting performance in the species. Seeds were subjected to six stratification durations (0, 15, 30, 45, 60 and 75 days) and four temperature treatments to break the dormancy. Stratification of 45 to 60 days as outdoor-pit treatment (16.5°/4.5°C:d/n) followed by 4±1°C temperature accelerates the germination along with significant increase in reducing sugar, total sugar and soluble protein while decreasing the total starch content in stratified seeds.

Key words: **Biochemical changes, dormancy, germination, *Pinus gerardiana*, stratification**

INTRODUCTION

Pinus gerardiana Wall. commonly known as chilgoza or neoza pine grows in dry temperate zone covering Kinnaur, Pangi, Bharmour and Kishtwar area of north-west Himalaya between 1600-3300 m elevation above mean sea level (latitude 30°-37°N and longitude 66°-88°E). The species is valued for its edible nut, which fetches very high price ranging from Rs 400-600/kg and therefore plays an important role in the economic uplift of the people of Himachal Pradesh and Jammu and Kashmir. Chilgoza pine has aptly been described as the champion of the species Rocky Mountains as it grows under difficult site conditions prevailing in the inner Himalaya. There is thus an urgent need to undertake large scale afforestation. Chilgoza pine has aptly been described as the champion of the species. The conifer seeds in general have a high degree of dormancy which prevents their germination even when provided with favourable environmental condition (Thapliyal and Gupta 1980, Jull and Blazich 2000, Sharma 2005). The dormancy may primarily be due to under developed embryo or decreased metabolic activity in the seeds. The stratification treatment enables the induction/activation of enzymes, hormones and other soluble metabolites needed by embryo for germination (Abdul-Baki and Anderson 1970, Blanche *et al*. 1990, Dogra 2003). The present study was therefore undertaken to investigate the effect of stratification and temperature on germination and germination associated biochemical parameters in chilgoza seeds.

MATERIALS AND METHODS

The present study was conducted in the Department of Silviculture and Agroforestry, Dr. YSPUHF, Nauni-Solan during 2004-06. The freshly collected seeds were subjected to six periods of stratification treatment at fifteen days interval i.e., P_1 (0 day-control), P_2 (15 days), P_3 (30 days), P_4 (45 days), P_5 (60 days) and P_6 (75 days)

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under four temperature regimes, viz., T_1 (room temperature:19°-22°C), T_2 (out-door pit: 16.5°/4.5°C), T_3 (4±1°C) and T_4 (-4±1°C) in moist sand. The germination was recorded daily up to 28 days to determine the germination per cent of seeds. The experiment thus comprised twenty four treatments of 90 seeds/plot each replicated thrice in completely randomized block design (factorial) in germinator at 25±1°C. The moisture content of seeds was determined by Toluene distillation method (ISTA 1966) as follows-

$$\text{Moisture content (\%)} = \frac{\text{Original weight – Oven dry weight}}{\text{Original weight}} \times 100$$

The dried sample of each treatment was used to determine the biochemical parameters of the seeds as follows. One gram of dried sample was placed in 20-25 ml of boiling ethanol (80%) for 10 minutes and decanted. Another 10-15 ml of boiling ethanol was added to the residue. Thereafter, the two extracted samples were filtered and combined and the final volume made to 50 ml. The alcoholic extract was then used for the estimation of total sugars while the residue used for determination of starch. The total sugars were estimated by phenol-sulphuric acid method given by Dubois et *al*. (1951). The reducing sugar was quantified by di-nitrosalicylic acid method developed by Miller (1972). The content of non-reducing sugars was calculated by deducting the quantity of reducing sugars present in the individual sample from that of the total sugar present and then multiplied by a factor (0.95). Similarly, the glucose in the sample was determined by phenol-sulphuric acid method of Dubois *et al*. (1951) and starch content calculated by multiplying the glucose value with conversion factor (0.90). The soluble proteins were estimated by the method prescribed by Lowry *et al*. (1951). The research uses two years data (2004-05 and 2005-06) which was pooled and then analyzed statistically as per Gomez and Gomez (1984). The least significant difference at 5 per cent level was used to test the significant differences among various treatments. Data as percentages were transformed to arcsine $(x/100)^{0.5}$.

RESULTS AND DISCUSSION

Effect of stratification period: The pooled data presented (Fig. 1) reveal that stratification period exert

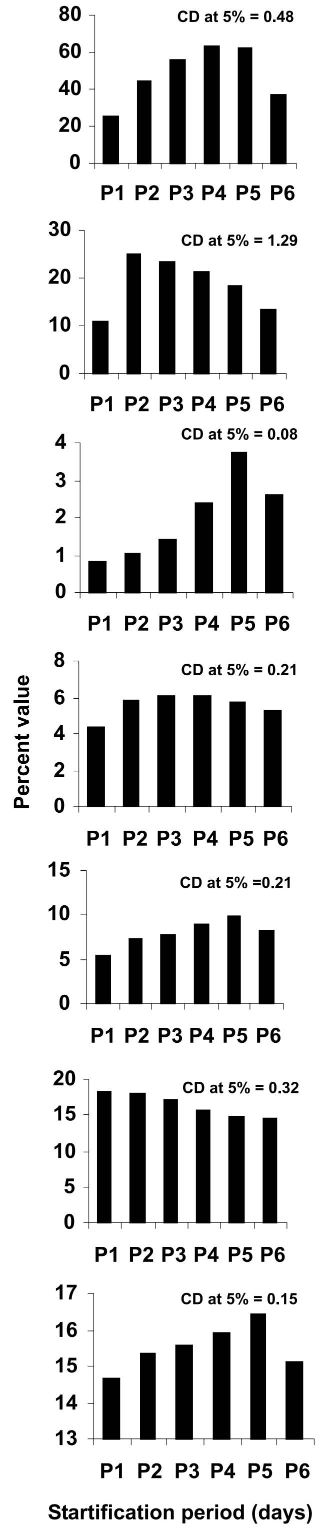

Fig. 1. Effect of stratification period on (a) germination per cent, (b) moisture percent, (c) reducing sugar, (d) non- reducing sugar, (e) total sugar, (f) starch and (g) soluble proteins.

a significant effect on germination (Fig. 1a), moisture content (Fig. 1b), reducing sugar (Fig. 1c), non-reducing sugar (Fig. 1d), total sugar (Fig. 1e), starch (Fig. 1f) and soluble protein (Fig. 1g) contents of seeds. The highest germination of 63.30 per cent resulted when seeds were stratified for 45 days (P_4), the results being statistically at par with P_5 (60 days) which exhibit 62.28 per cent success. The P_4 stratified seed treatment enhanced germination by 60.26 per cent more as compared to non-stratified (control) seeds. Singh (1989) on the other hand showed that stratification of 60 days resulted in 19.75 per cent more germination in spruce over that obtained in non-stratified seeds.

It was evident (Fig. 1b) that moisture content increased up to P_2 treatment and thereafter decreased as the stratification period was increased from 15 days to 75 days. The significantly maximum moisture content (25.14%) was observed for 15 days (P_2). The least value (10.95%) was recorded in unstratified control (P_1) seeds. The reducing sugar (3.75%), total sugar (9.83%) and soluble protein (16.41%) were significantly increased when seeds were stratified for 60 days (P_5). It is clearly indicated that biochemical constituents are changed during stratification. Similar results have also been reported by Ching (1973) and Dogra (2003). The significantly maximum non-reducing sugar (6.10%) resulted when seeds were stratified for 45 days (P_4). The peak in reducing sugar, total sugar and soluble proteins coincides with high germination in seeds and therefore is considered as effective markers to determine seed vigor in chilgoza pine. Gautam (1997) reported that soluble proteins increased from 30 days to 75 days of stratification and thereafter showed a declining trend up to 120 days in *Quercus leucotrichophora*. Our results are thus in agreement with that of Sofi and Bhardwaj (2007) in *Cedrus deodara* seeds who reported that soluble proteins and total sugar increased from 15 days to 60 days of stratification and thereafter exhibit a decreasing trend. The non-stratified (control) seeds were found to exhibit least significant values for all these biochemical constituents which coincides with least germination (25.09%) in the seeds. Contrary to this, however the starch content exhibits a declining trend throughout as the stratification period was increased from 0 to 75 days.

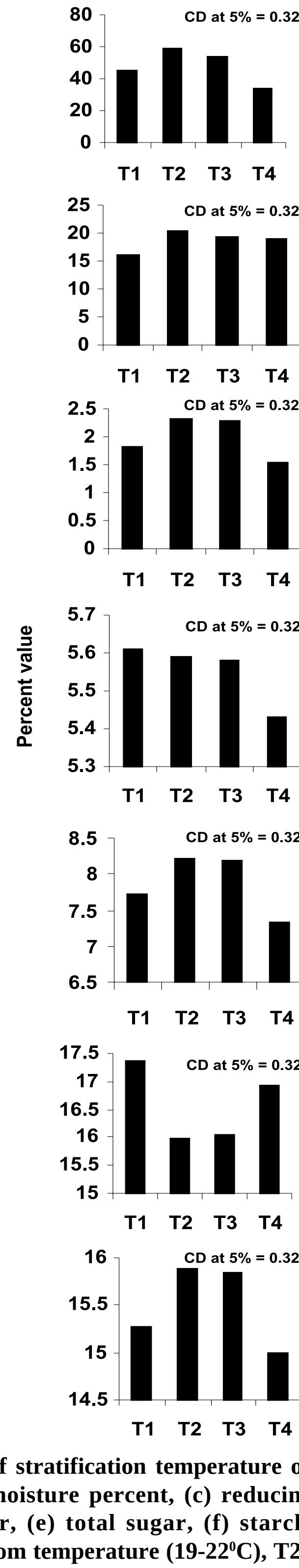

Fig. 2. Effect of stratification temperature on (a) germination per cent, (b) moisture percent, (c) reducing sugar, (d) non-reducing sugar, (e) total sugar, (f) starch and (g) soluble proteins. T1 room temperature (19-22^{0}C), T2 outdoor pit (16.5/4.5^{0}C), T3 4±1^{0}C and T4 (-4±1^{0}C).

The maximum starch content (18.36%) was recorded in non-stratified seeds (P_1), while the minimum (14.49%) resulted when seeds were stratified for 75 days (P_6). The least value of starch with increasing stratification period indicated its break down in to simpler form of sucrose and monosaccharide contents which therefore results in releasing of dormancy in chilgoza seeds.

Effect of stratification temperature: It is evident from the (Fig. 2) that stratification temperature exerts significant effect on germination, moisture content and biochemical parameters in chilgoza seeds. The data indicated that maximum germination of 59.15 per cent (Fig. 2a), moisture content of 20.33 per cent (Fig. 2b), reducing sugar of 2.32 per cent (Fig. 2c), total sugar of 8.21 per cent (Fig. 2e) and soluble proteins of 15.88 per cent (Fig. 2g) resulted when seeds were stratified as out door-pit treatment (T_2). However, the minimum values of all these attributes were obtained when seeds were stratified at -4±1°C (T_4). On the other hand, non-

Table 1. Interaction effect of stratification period and temperature (PxT) on germination, moisture and bio-chemical parameters of *Pinus gerardiana* seeds under laboratory conditions.

Treatments (PxT)	Period of treatments	Germination (%)	Moisture content (%)	Reducing sugar (%)	Non-reducing sugar (%)	Total sugar (%)	Starch (%)	Soluble protein (%)
P_1T_1	control	25.09 (30.04)	10.95	0.81	4.38	5.43	18.36	14.65
P_2T_1	15d/RT(19°-22°C)	42.51 (40.62)	22.82	0.91	5.89	7.09	18.23	15.16
P_3T_1	30d/RT(19°-22°C)	56.38 (48.69)	20.64	1.09	6.15	7.57	17.83	15.29
P_4T_1	45d/RT(19°-22°C)	54.72 (47.72)	17.61	1.96	5.82	8.10	16.85	15.65
P_5T_1	60d/RT(19°-22°C)	57.25 (49.24)	15.07	3.27	5.73	9.31	16.57	15.85
P_6T_1	75d/RT(19°-22°C)	36.48 (37.04)	13.10	2.80	5.69	8.81	16.35	15.03
P_2T_2	15d/OD(16.5°/4.5°C)	55.30 (48.10)	26.30	1.09	5.83	7.23	17.99	15.78
P_3T_2	30d/ OD(16.5°/4.5°C)	65.74 (54.23)	23.11	1.58	6.09	8.00	16.47	15.92
P_4T_2	45d/ OD(16.5°/4.5°C)	83.42 (66.34)	21.30	2.63	6.23	9.20	14.95	16.37
P_5T_2	60d/ OD(16.5°/4.5°C)	79.35 (63.50)	17.57	4.71	5.67	10.69	13.38	17.00
P_6T_2	75d/ OD(16.5°/4.5°C)	45.73 (42.52)	14.77	3.09	5.31	8.68	13.34	15.55
P_2T_3	15d/(4±1°C)	44.99 (42.05)	26.71	1.15	5.83	7.30	18.04	15.75
P_3T_3	30d/(4±1°C)	63.33 (52.91)	24.91	1.67	6.14	8.14	16.54	15.84
P_4T_3	45d/(4±1°C)	74.82 (60.25)	23.87	2.72	6.18	9.24	15.01	16.21
P_5T_3	60d/(4±1°C)	71.89 (58.40)	20.64	4.66	5.61	10.68	13.69	16.99
P_6T_3	75d/(4±1°C))	42.96 (40.94)	14.89	2.82	5.40	8.51	13.56	15.59
P_2T_4	15d/(-4±1°C)	35.83 (36.58)	24.74	0.87	5.88	7.07	18.15	14.73
P_3T_4	30d/(-4±1°C)	39.63 (38.90)	24.24	1.38	5.79	7.60	17.73	15.19
P_4T_4	45d/(-4±1°C)	40.55 (39.46)	22.28	2.13	6.16	8.62	16.05	15.37
P_5T_4	60d/(-4±1°C)	40.38 (39.40)	19.67	2.36	5.83	8.65	15.33	15.80
P_6T_4	75d/(-4±1°C)	23.07 (28.65)	14.29	1.58	4.55	6.56	14.35	14.23
SEm ±	-	**0.49**	**1.28**	**0.08**	**0.21**	**0.21**	**0.32**	**0.31**
$CD_{0.05}$	-	**1.04**	**NS**	**0.16**	**0.44**	**0.44**	**0.68**	**NS**

Figures in parentheses are arcsine transformed values
d= days, RT= Room temperature, OD= out-door temperature

reducing sugar and starch content exhibit a different trend with maximum value of 5.61 per cent and 17.36 per cent respectively (Fig. 2d & 2f) when the seeds were stratified at room temperature (T_1). Our results coincides with the Leadem (1986) who reported that moderate (15/10°C: day/night) moist stratification condition produced complete germination in *Abies amabilis* seeds than warm (30/20°C) conditions. Schineider and Gifford (1994) on the other hand reported that moist stratification of 35 days at 2°C increased seed germination from 19 to 76 per cent in *Pinus taeda*. Similar results have also been reported by many other research workers like Janice *et al*., (2002) and Kao and Rowan, (2005).

Interaction effect of stratification period and temperature (PxT): The pooled data pertaining to interaction effect of stratification period and temperature (PxT) reveals significant influence on germination, moisture content and biochemical parameters, viz., reducing sugar, non reducing sugar, total sugar and starch content (Table 1). The highest germination of 83.42 per cent was obtained when seeds were stratified for 45 days as outdoor-pit (P_4T_2) treatment. This was however, followed by treatment combinations P_5T_2, P_4T_3 and P_5T_3 giving values of 79.35 per cent, 74.82 per cent and 71.89 per cent respectively. The minimum of 23.07 per cent resulted when seeds were stratified for 75 days at -4±1°C (P_6T_4). The significantly maximum reducing sugar (4.71%) and total sugar (10.69%) resulted when seeds were stratified for 60 days as outdoor pit treatment (P_5T_2). The minimum value of 0.81 per cent and 5.43 per cent respectively was registered in non-stratified seeds kept at room temperature (P_1T_1). The bio-chemicals thus trend to coincide with the peak of germination success of the species. According to Sharma (2005), the germination success in *Pinus gerardiana* can be improved by subjecting the seeds to 20 weeks of moist chilling treatment. However, it is clearly indicated from our results that moist chilling period of 45 to 60 days would be sufficient for better germination success in the species when stratified at out-door pit (16.5°/4.5°C) or 4±1°C temperature. On the contrary, while significantly maximum starch content of 18.36 per cent was observed in control (P_1T_1), the minimum value (13.34%) resulted when seeds were stratified as P_6T_2, the latter being at par with P_5T_2 (13.38%) and P_5T_3 (13.69%) in this regard. Though non-significant, the maximum soluble protein (17.0%) was recorded when seeds were stratified for 60 days as outdoor pit P_5T_2 treatment. Our results are supported by Kao and Rowan (2005) who indicated that stratification of *Pinus radiata* at 0°C increased organic acids, sucrose and organic phosphate in the seeds which accelerated subsequent germination in the species. Similar trends have also been reported by Dogra (2003) in silver fir and spruce.

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