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Influence of Mechanical Scarification and Gibberellic Acid on Seed Germination and Seedling Performance in *Pinus gerardiana* Wall

Lokinder Sharma[1], B. Madhusudhana Reddy[1,2]*, Moumita Chatterjee[2], Salil Dhawan[1] and Vinayak Pai[2]

[1]*Forest Research Institute Deemed to be University, Dehradun, India*
[2]*Institute of Wood Science and Technology, Bengaluru, India*

**Corresponding author*

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Pinus gerardiana is commonly called "Chilgoza Pine" which yields small edible seeds. The pine is a vital ecological and economic forestry species having restricted distribution in India. It's mostly found in two districts of Himachal Pradesh. It's been observed that the natural regeneration of the species is extremely poor or entirely lacking. This species has erratic and infrequent seed years and dormancy related problems that also reduce the regeneration process in natural habitats. Therefore, different treatments viz. Mechanical scarification, Gibberellic acid (100ppm) and combination of both, where the soaking period is kept 24hours and incubation temperature is kept $25^0C\pm1^0C$. The seeds were counted as germinated when radicle was a minimum of two mm long. The effect of pre-sowing treatments on various germination parameters (Germination %, Germination energy, mean daily germination, Peak value, Germination value, Germination index) was calculated and interpreted. After this, germinated seeds were put in root trainers and allowed to grow within the net house. After the establishment of seedlings various seedling assessment parameters (collar diameter, root-shoot length, number of needles) were measured properly for every treatment. Seed Vigour Index was also calculated separately. This study identified T_4 (Mechanical Scarification + 100ppm Gibberellic acid) treatment as the best treatment for enhancing germination and seed vigour.

Introduction

Pinus gerardiana Wall is a small to a moderate-sized evergreen tree, varying from 2 to 4 m in girth and 17 to 27 m in height (Bhattacharrya, 1988). Branches somewhat ascending and usually not whorled. The bark is thin, grey, exfoliating in irregular thin flakes which leave shallow depressions. Leaves needle-like, dark green and exists in clusters of three. Chilgoza seed is cylindrical, pointed, dark brown, with rudimentary wings, 2 - 2.5 cm long, endosperm oily and edible (Luna, 2008). The species has been described as the "Champion of Rocky Mountains" because it grows under extremely rough site conditions and may withstand the extremes of cold climate and aridity. Chilgoza is found only within the inner arid valleys of the North-West Himalayas, where the rainfall is scanty but there's heavy snowfall, the total precipitation amounting between 370-750mm. It endures severe cold in winter, the summer temperature within its habitat seldom exceeds 37^{o}C (Luna, 2008). This species is sparsely distributed in Himachal Pradesh, covering a complete area of about 2060 ha with most of the area falling in Kinnaur District (2040 ha) and a small portion (20 ha) in Chamba district of Himachal Pradesh (Troup, 1921). According to the IUCN list 2020, this species belongs to a Near-threatened category.

It's a really important species from an ecological and economical point of view. The species is found within the dry temperate region which is extremely fragile, where landslide and erosion is a big problem during the rainy season. The tree is extremely much adapted to the present region, thanks to its inherent capacity to grow on rough terrain, even on a bare rock which has shallow soil, this tree is named as the champion of rock.

The seeds and seed oil of the plant have medicinal properties; the seeds are used as anodyne and stimulant. The oil is additionally used against wounds and ulcers. It's the sole conifer species in North West Himalayas that gives edible Kernels/nuts rich in carbohydrate, fat, fiber and mineral matter. Being nutritious and delicious, the seeds have high demand in local, national and international markets and fetch excellent prices.

Due to these high-priced edible nuts, this tree is being overexploited. This overexploitation affects the regeneration of this tree, where every cone is taken away for profit maximization and nothing is left for natural regeneration. All these activities adequately affect the seed germination behavior and seed vigour of the species.

The study of *Pinus gerardiana* germination is important for several reasons. First, our knowledge regarding *P. gerardiana* germination is inadequate, so it's important to know the germination pattern within the species. Second, *P. gerardiana* is a dominant component of dry temperate forests; the reduction of area under species would adversely affect ecology and economy of the region (Urooj and Jabeen, 2015).

The area under species has declined from 2500 ha (Singh, 1992) to 2040 ha (Sharma *et al.*, 2010) in the Kinnaur region. Pine forests play a crucial ecological role in regulating rivers and streams that originate and flow in a particular region (Harmon *et al.*, 1986; Bilby and Bisson, 1998), and *P. gerardiana* helps in watershed protection in Himalayas (Akbar *et al.*, 2014).

Because of all these major ecological roles played *by P. gerardiana* in dry temperate forests of Himalayas, it is essential to know the functioning of processes within these forests systems. Third, the construction of hydroelectric projects and other development activities (Yadav, 2009) had led to reduction of species distribution which will adversely affect the ecology of the region (Sarkar, 2008); for future management of those forests, it is essential to get the basic knowledge about the germination of this species.

Pre-sowing seed treatments have attracted considerable attention in recent years due to its role in enhancing germination and subsequent growth of the seedling. Besides the long duration stratification process, efficient pre-sowing treatments envisaging the soaking of seeds for a few minutes to days in growth regulators or water have been tried to break dormancy. The study has the following objectives such as to find out the best pre-sowing treatment for the germination and to assess the various growth characteristics of seedlings like collar diameter, number of leaves per seedling, root and shoot length to determine the Seed Vigour Index (S.V.I) for different treatments.

Materials and Methods

Study area

The present study was conducted in the seed technology laboratory, Silviculture Division and Central Nursery of Forest Research Institute, Dehradun, Uttarakhand, India. The study work started in February 2016 and completed until mid-May 2016. The seed source of *P. gerardiana* was Luj, Pangi Forest Division, Chamba, Himachal Pradesh, India (Fig. 1). The area lies between 33^{0}7'37.60 N latitude and 76^{0}20'1.9 E longitude.

Champion and Seth (1968) recognize ten subtypes of the forest in the Pangi region. Out of those ten subtypes, one is Neoza Pine Forest comprises species *Pinus geradiana, Cedrus deodara, Fraxinus floribunda, Celtis australis* in the dry and rocky areas in Luj and Kanun Reserve forest. The elevation of the Pangi valley ranges from 2006 to 6168m MSL (Kumar *et al.,* 2014).

Pre-sowing treatment

The seed coat of *P. gerardiana* is slightly hard and impermeable which can prolong the germination period. So, it is necessary to offer pre-sowing treatment to these seeds. It's given with four different treatments with five replications each and CRD factorial design was adopted to see whether which treatment will give better germination. The treatments are as follows**,** T_1 = Control (Water Soaking)**,** T_2 = Mechanical Scarification and Water Soaking, T_3 = GA_3 (100ppm), T_4 = Mechanical Scarification and GA_3 (100ppm). The Soaking period was 24 hours. The Mechanical scarification is completed by fine sandpaper at an area opposite from the radicle initiation portion. The portion was rubbed until the megagametophyte was exposed.

Seed germination test

Seed germination study was conducted by taking 400 seeds and subjecting them to four treatments and each treatment has given five replications. The seeds were placed in Petri dishes and these kept in germinator having temperature 25°C ±1°C and humidity 100%. Seeds were checked regularly and counted as germinated when radicle was a minimum of 2 mm. Being a temperate species of high Himalaya testing period was kept as about 40 days.

Germination percentage

$$Germination\ (\%) = \frac{Number\ of\ seed\ germinated}{Total\ no.of\ seed\ kept\ for\ germination}\ X\ 100$$

Germination energy (GE %)

$$Germination\ energy(\%) = \frac{Number\ of\ seeds\ germinated\ up\ to\ the\ time\ of\ peak\ germination}{Total\ number\ of\ seeds\ sown}\ X\ 100$$

Mean daily germination (MDG) was calculated as the cumulative germination percentage of seeds at the end of the test period divided by the number of days from sowing to the end of the test or total days. Peak value (PV) was calculated as the maximum mean daily germination reached at any time during the period of the test (Czabator, 1962). Germination index (GI) was calculated by dividing the total number of seed germinated at the end of the experiment by the time taken for 50% germination. Germination value (GV) is the index combining speed and completeness of seed germination. Daily germination counts were recorded and calculated as per (Czabator, 1962).

GV = PV × MD
Where, PV = Peak value of germination, MDG = Mean daily germination

Analysis of variance (ANOVA)

The statistical analysis of each parameter was carried out on mean value and the analysis of variance was performed. The effect of treatment was assessed periodically through germination and initial growth performance of the seedlings in laboratory and root trainers respectively. Five seedlings from each treatment were randomly selected and uprooted very carefully to estimate the seedling length (i.e. root and shoot length), number of needles and collar diameter. Data were statistically analyzed for studying the morphological growth variation for each treatment (Table 2).

Seed vigour index (S.V.I)

Seed vigour is an important quality parameter that needs to be assessed to supplement germination and viability tests to gain insight into the performance of a seed lot in the field. The Seed vigour index (S.V.I) is calculated by multiplying germination (%) and seedling length. The seed lot showing the higher seed vigour index is considered to be more vigorous (Abdul-Baki and Anderson, 1973).

Results and Discussion

Effect of pre-sowing treatments

The data presented in Table 1 reveals that there is a significant difference between different pre-sowing treatments. The very best germination (67.00 %) was registered in T_4 treatment, at temperature $25^0C \pm 1^0C$ and soaking period 24 h. In a comparison of T_4 with $T_{1,}$ T_2 and T_3 treatment, the mean difference between T_4 and $T_{1,}$ T_4 and T_2, and T4 and T_3 is 43%, 14%, and 26% respectively. All these values are greater than the critical difference (CD) value (Fig. 2). This reveals that T_4 (Mechanical scarification + 100ppm GA_3) is significantly different from the rest of the treatments. Similar results were registered by Kumar et al. (2014), where germination was (73.84 and 62.71 %) when seeds were treated with Gibberellic acid concentration 75ppm and 150ppm respectively at temperature $25^{\circ}C$ and soaking period 24 h.

Germination energy (GE) (%) of seeds of T_4 (Mechanical scarification + 100ppm GA_3) treatment exhibit the highest germination energy (59.00%). However, this was followed by T_2 (Mechanical Scarification + Water) and T_3 (100ppmGA_3) (46.00% and 32.00% respectively) treatments in descending order. Minimum germination energy (19.00%) was, however, observed in T_1 (control) treatment. High germination with gibberellic acid treatment could be attributed to a rise in gibberellins in seeds during germination (Cetinbas and Koyunchu, 2006; Chen *et al.*, 2008; Dhoran and Gudadhe, 2012). Germination value (GV) of seeds which are mechanically scarified and then soaked in gibberellic acid was higher (3.70) than control (0.39) (Table 3).

The combined effect of scarification and 100ppm gibberellic acid resulted in an increase in GV of seeds than other treatment combinations. Gibberellic acid-treated seeds began germinating sooner and completed germination faster. It could probably be due to the facilitation of cytokinin penetration in the testa and neutralization of inhibitors present in the embryo, thus enabling the embryo to rupture the seed coat (Cetinbas and Koyuncu, 2006).

Seedling quality assessment is critical to ensure reforestation success. While height and collar diameter is that the commonest traits evaluated during the seedling quality assessment, above ground morphology is not an accurate predictor of performance after out-planting. Root system morphology status may provide a more accurate indication of seedling potential (Anthoney *et al.*, 2005). In light of the above reference, various traits of seedling quality were measured and their mean value is mentioned in Table 4.

Maximum mean collar diameter (2.36mm) was registered in T_4 treatment, the maximum mean number of needles (14.60) was found in T_2 treatment, maximum root and shoot length (6.9 and 7.7cm respectively) was found in T_4 treatment. Lowest seedling mean collar diameter, shoot length and number of needles (2.07mm, 6.9cm, and 11.80 respectively) were found in T_1 treatment. Whereas the lowest root length (6.1cm) was found in T_3 treatment. Seed Vigour Index (S.V.I) was calculated by using the above-given formula.

The seed treatment showing the higher seed vigour index is considered to be more vigorous (Abdul-Baki and Anderson, 1973). Maximum S.V.I (978.20) was observed in T_4 treatment, after that T_2 and T_3 (752.60 and 557.60 respectively) in descending order. Lowest S.V.I (321.60) was observed in T_1 treatment. Rubbing the basal portion of seed which exposes the megagametophyte resulted in the promotion of the final germination of *P. gerardiana*. Table 3 clearly shows that T_4 and T_2 (Mechanically scarified) treatments show the highest germination as compared to T_3 andT_1 (non-scarified). The rate of final germination in intact seeds of *P. gerardiana* was very slow, however, a remarkable increase both in rate and final germination was observed when the seeds were imbibed after seed coat removal. The reason why this enhancement occurs is not fully understood.

The seed coat of *P. gerardiana* is thin and papery and readily permeable to water. Therefore, there is no possibility of it interfering with water uptake. It may act as a barrier to gaseous exchange in the first few days of imbibition when the seed is in the activation stage with increasing requirement of oxygen.

The possible role of seed coat as an obstacle for germination may be due to the presence of certain chemical inhibitors in the seed coat or it may act as a barrier against the leaching out of inhibitors present inside the seed. The evidence that pine seed coat has water-soluble germination inhibitors was presented by some workers in the seed coat of *Pinus pinea*, they suggested that those germination inhibitors were involved in the regulation of *P. pinea* seed germination (Martinez *et al.*, 1978). In light of the possibilities discussed above, there is a need to further explore this aspect of *P. gerardiana* seeds (Fig. 3-10).

Table.1 Germination % of different treatments and replications

Treatment	Replication					Total	Germination %
	R_1	R_2	R_3	R_4	R_5		
T_1 (control)	15	20	40	25	20	120	24
T_2 (Scarification + Water Soaking)	65	50	45	55	50	265	53
T_3 (100ppm GA_3)	35	40	25	60	45	205	41
T_4 (Scarification + 100ppm GA_3)	75	55	70	75	60	335	67
Total	190	165	180	215	175	925	

Table.2 Analysis of Variance (ANOVA)

Source of variation	df	SS	MSS	F calculated	F tabulated
Treatment	3	4993.75	1664.58	16.64	3.24
Error	16	1600.00	100.00		
Total	19	6593.75	347.03		

$CD_{0.05}$
Critical difference for Germination (%) = 13.39

Table.3 Effect of Pre-sowing treatments on Germination Value, Mortality %, Germination energy and Germination Index

Treatment	Germination Value	Mortality %	Germination energy (GE %)	Germination Index
T_1	0.39	76	19	1.09
T_2	1.97	47	46	2.41
T_3	1.07	59	32	1.64
T_4	3.70	33	59	3.72

Table.4 Mean value of seedlings growth characteristics and Seed Vigour Index

Treatment	Collar Diameter (mm)	No. of needles	Root Length (cm)	Shoot Length (cm)	Seedling Length (cm)	S.V. I
T_1	2.07	11.80	6.5	6.9	13.4	321.60
T_2	2.26	14.60	6.8	7.4	14.2	752.60
T_3	2.11	12.20	6.1	7.5	13.6	557.60
T_4	2.36	14.20	6.9	7.7	14.6	978.20

Fig.1 Map showing seed source area

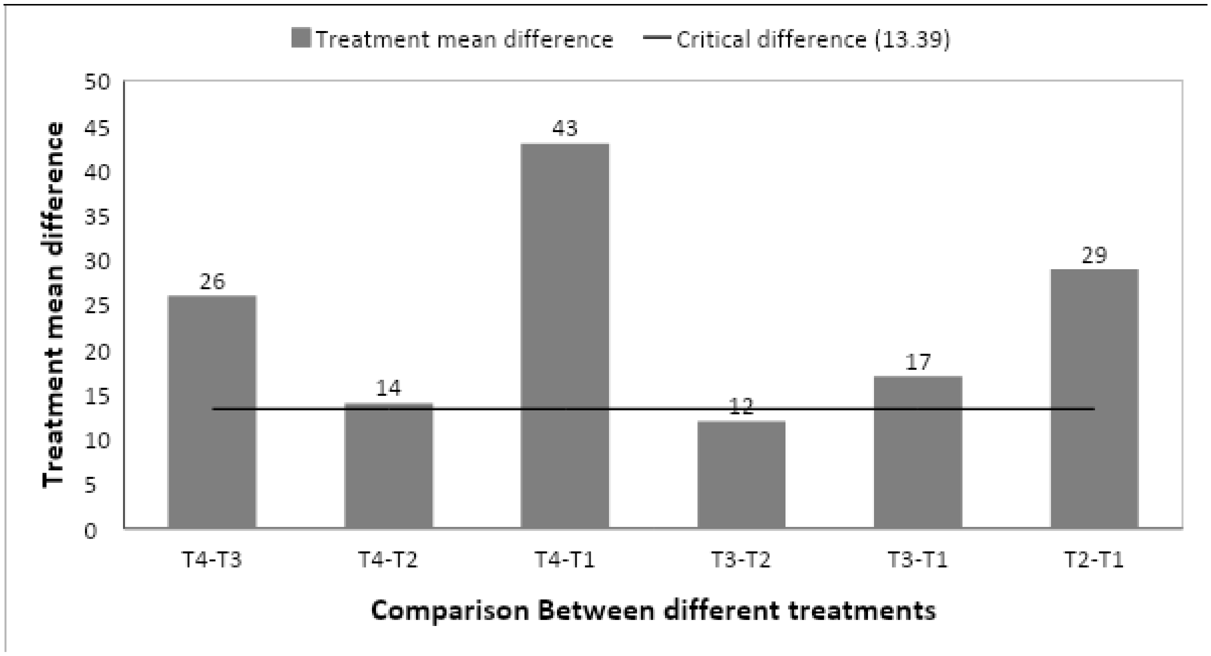

Fig.2 Significant difference between different treatments

Fig.3 Different Treatments given to Seeds

Fig.4 Cleaning of Seeds attacked by Fungus

Fig.5 First sign of germination

Fig.6 Germination at Full Capacity

Fig.7 Mechanical Scarification

Fig.8 Measurement of Seed Traits

Fig.9

Fig.10

The results of the study conclude that germination and seedling growth of *P. gerardiana* seeds depends on pre-sowing treatments. From the study, it is recommended that a combination of T_4 (100 ppm gibberellic acid + Mechanical scarification), 24 h soaking and $25^0C \pm 1^0C$ incubation temperature is best for enhancing germination and seedling growth of Chilgoza pine.

As there is no significant difference between T_2 (scarification + water soaking) and T_3 (100ppm GA_3), both these treatments are statistically the same. So, it is also recommended to prefer T2 (scarification + water soaking) over T_3 (100ppmGA_3) because this will reduce the cost of production of Chilgoza seedlings in the nursery. The reason why this enhancement occurs in mechanically scarified seeds is not fully understood.

The possible reason may be a seed coat act as a barrier against the leaching out of inhibitors present inside the seed. However, extensive research on the effect of seed coat could further elucidate protocol for faster germination and seedling growth of *P. gerardiana*.

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