International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(1): 1635-1638

Received: 20-11-2018
Accepted: 23-12-2018

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Effects of different seed treatments on germination of endangered *Pinus gerardiana*

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Abstract
The study was conducted on Endangered *Pinus gerardiana* (Chilgoza Pine) which has restricted distribution in India. It has been observed that the natural regeneration of the species is extremely poor or entirely lacking, since the species has erratic and infrequent seed years and dormancy related problems. Therefore, different treatments viz. Mechanical scarification, Gibberellic acid (100ppm) and the combination of both were given, where a soaking period is kept 24h and incubation temperature is kept 25 ^{0}C±1 ^{0}C. The seeds were counted as germinated when radicle was at least 2 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 in net house. After the establishment of seedlings various seedling assessment parameters (collar diameter, root-shoot length, number of needles) were measured properly for each treatment. Seed Vigour Index was also calculated separately. The present study identified T_4 (Mechanical Scarification + 100ppm Gibberellic acid) treatment as the best treatment for enhancing germination and seed vigour.

Keywords: *Pinus gerardiana,* Chilgoza, edible, endangered, germination, gibberellic acid

Introduction
The only edible Pine of India is on the verge of extinction. Yes, Chilgoza Pine is an endangered tree species of western Himalayas. It is a very important species from an ecological and economical point of view. Due to its inherent capacity to grow on rough terrain, even on a bare rock which has shallow soil, this tree is called a champion of rock. Simultaneously it also provides edible nuts in this type of area where food is the main limiting factor for the survival of wild animals and birds. Due to high prices of these 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 essential for many reasons. First, our knowledge regarding *P. gerardiana* germination is lacking, so it is important to understand germination pattern in 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) [1]. The area under species has declined from 2500 ha (Singh 1992) [2] to 2040 ha (Sharma *et al*. 2010) [3] in Kinnaur region. Pine forest play an important ecological role in regulating river and streams that originate and flow in a particular region (Harmon *et al*. 1986; Bilby and Bisson 1998) [4, 5], and *P. gerardiana* helps in watershed protection in Himalayas (Akbar *et al*. 2014) [6]. Because of all these major ecological roles played *by P. gerardiana* in dry temperate forests of Himalayas, it is essential to understand the functioning of processes within these forests systems. Third, the construction of hydroelectric projects and other development activities (Yadav 2009) [7] had led to the reduction of species distribution that will adversely affect the ecology of the region (Sarkar 2008) [8]; for future management of these forests, it is essential to get basic knowledge about the germination of this species. Therefore, the present study was conducted to find out the best pre-sowing treatment for the germination of *Pinus gerardiana,* to assess the various parameters of seedlings like collar diameter, number of leaves per seedling, root and shoot length, to find out the Seed Vigour Index (S.V.I) for different treatments and along with this to show the enormous potential of germination of seeds of *P. gerardiana* of Pangi Forest Division, Chamba District, Himachal Pradesh.

Materials and Methods

Study area

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

Treatments- 4
Replications- 5
Seeds number- 100 seeds per treatment
Design – Completely Randomized Design (CRD) Factorial

Morphological study of seed

In this study, various dimensions of seeds were measured and the mean value of each dimension was calculated. In this seed length, seed thickness and seed width are measured from ungraded seed lot and their mean value is also calculated separately. The weight of a hundred seeds was also measured (Table 1).

Pre-sowing treatment of seeds

The seed coat of *P. gerardiana* is slightly hard and impermeable which may prolong the germination period. So it is necessary to give pre-sowing treatment to these seeds. I gave four different treatments to check whether which treatment will give better germination

T_1 = Control (Water Soaking), T_2 = Mechanical Scarification + Water Soaking, T_3=GA_3 (100ppm) and T_4= Mechanical Scarification + GA_3 (100ppm)**.** The seeds were soaked for 24 hours for each treatment. The Mechanical scarification is done by fine sandpaper at an area opposite from the radicle initiation portion. The portion was rubbed until the megagametophyte was exposed. To ensure the smooth germination of seeds it is inevitable to protect the seeds from fungus attack. For this purpose pre-treatment of Bavistin (0.1%) is given. In this, seeds were soaked in Bavistin solution for about an hour.

Seed germination

Total 400 seeds were divided into four treatments and each treatment has given five replications. Now, these seeds were placed in Petri dishes precisely without disturbing much. Proper distance is maintained between each seed to avoid contamination from one seed to another seed. After this, each petri dish is marked separately according to its treatment and replication. These Petri plates were then kept in germinator having temperature 25ºC ±1ºC and humidity 100%. Seeds were checked regularly and necessary precautions were taken to retard fungus growth. In the case of seed contaminated with fungus, it is cleaned by using alcohol. Watering was done regularly to keep the germination paper moist. But an excess of watering was also avoided because. it stimulates fungus growth. The very first sign of germination was shown by T_2 (Mechanical Scarification + Water Soaking) after about a week. The seeds were counted as germinated when radicle was at least 2 mm. As being a temperate species of high Himalaya testing period was kept as about 40 days (Figure 1).

Fig 1: Different stages of Chilgoza Seed germination

Germination percentage

The object of testing the germinative quality of seed is to provide an indication of the percentage of seeds in a given lot that is expected to produce seedlings. It also provides a comparison for a quality index for one seed lot with another of the same species and with another. The percent germination was calculated by a number of seeds in a given sample that actually germinated (Table 2).

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

Mean daily germination

Mean daily germination 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 (Figure 2 and Table 3).

Peak value

Peak value was calculated as the maximum mean daily germination reached at any time during the period of the test (Czabator, 1962) [9] (Table 3)

Germination value

Germination value is the index combining speed and completeness of seed germination. Daily germination counts were recorded and calculated as per (Czabator, 1962) [9] (Table 3)

GV = pv × mdg

Where,

pv = Peak value of germination
mdg = Mean daily germination

Germination energy

Germination energy is a measure of the speed of germination and hence is the indicator of the vigour of seed and seedling. Germination energy was calculated on the basis of the percentage of the total number of seeds that had germinated when the germination reached its peak (Table 4).

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

Germination index

Germination index was calculated by dividing the total number of seed germinated at the end of the experiment by the time is taken for 50% germination (Table 4).

Shifting of germinated seeds to root trainers and assessment of seedlings

Root and shoot length, number of needles and collar diameter were analyzed after transplantation of seedlings to root trainers (Table 5).

Results

Table 1: Mean value of seed traits recorded

S. No	Seed Traits	Mean Value
1.	Seed length (mm)	20.49
2.	Seed width (mm)	06.74
3.	Seed thickness (mm)	05.45
4.	Weight of 100 seeds (g)	37.92

Table 2: 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	

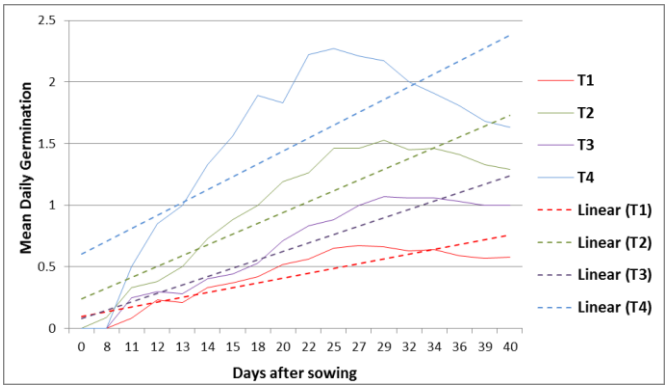

Fig 2: Mean Daily Germination

Table 3: Effect of Pre-sowing treatments on Peak value, Mean Daily Germination, Germination Value, and Mortality %

Treatment	Peak Value	Mean Daily Germination	Germination Value	Mortality %
T_1	0.67	0.58	0.39	76
T_2	1.53	1.29	1.97	47
T_3	1.07	1.00	1.07	59
T_4	2.27	1.63	3.70	33

Table 4: Effect of Pre-sowing treatment on Germination energy and Germination Index

Treatment	Germination energy (ge %)	Germination Index
T1	19	1.09
T2	46	2.41
T3	32	1.64
T4	59	3.72

Table 5: Mean value of seedlings growth characteristics

Treatment	Collar Diameter (mm)	No. of needles	Root Length (cm)	Shoot Length (cm)
T_1	2.07	11.80	6.5	6.9
T_2	2.26	14.60	6.8	7.4
T_3	2.11	12.20	6.1	7.5
T_4	2.36	14.20	6.9	7.7

Seed vigour index (S.V.I)

Table 6: Seed Vigour Index (S.V.I)

Treatment	Seedling Length (cm)	Germination %	S.V.I
T_1	13.4	24	321.60
T_2	14.2	53	752.60
T_3	13.6	41	557.60
T_4	14.6	67	978.20

Discussion

It is evident from Table 2, that the germination % obtained under the influence of control is low as compared to mechanical scarification and Gibberellic acid. The highest germination (67.00%) was registered in T_4 treatment, at temperature 25 ^{0}C ± 1 ^{0}C and soaking period 24 h. Similar results were registered by Kumar *et al*. (2014) [11], where germination was (73.84 and 62.71%) when seeds were treated with Gibberellic acid concentration 75ppm and 150ppm respectively at temperature 25 ^{0}C and soaking period 24 h.

Germination value (GV) and Mean Daily Germination of seeds which are mechanically scarified and then soaked in gibberellic acid was higher than control (Table 3 and Figure 2). The combined effect of scarification and 100ppm gibberellic acid resulted in an increase in GV of seeds than other treatment combination. 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) [12].

Germination energy (GE) (%) of seeds which are mechanically scarified and then soaked in gibberellic acid (100 ppm) was greater than control (Table 4). High germination with gibberellic acid treatment might be attributed to an increase in gibberellins in seeds during germination (Cetinbas and Koyunchu 2006; Chen *et al*. 2008; Dhoran and Gudadhe 2012) [12, 13, 14] (Table 4).

Seedling quality assessment is critical to ensure reforestation success. While height and collar diameter are the most common 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 Davis *et al*. 2005 [14]. In the light of the above reference, various traits of seedling quality were measured and their mean value is mentioned in Table 5. 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) was found in T_1 treatment. Whereas the lowest root length (6.1cm) was found in T_3 treatment. Now the Seed Vigour Index (S.V.I) is calculated. The seed treatment showing the higher seed vigour index is considered to be more vigorous (Abdul-Baki and Anderson, 1973) [15]. Maximum S.V.I (978.20) is calculated for 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) is calculated for T_1 treatment (Table 6).

Rubbing the basal portion of seed which exposes the megagametophyte resulted in the promotion of final germination of *P. gerardiana*. Table 2 clearly shows that T_4 and T_2 (Mechanically scarified) treatments show the highest germination as compared to T_3 andT_1 (non-scarified). Saeed

and Thanos (2006) [16] also observed that the rate of final germination in intact seeds of *P. gerardiana* was very slow; however 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. In the light of the possibilities discussed above, there is a need to further explore this aspect of *P. gerardiana* seeds.

Conclusion

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 ($100ppmGA_3$), both these treatments are statistically the same. So it is also recommended to prefer T_2 (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 seed coat act as a barrier against the leaching out of inhibitors present inside the seed. However additional research on the effect of seed coat could further elucidate protocol for faster germination and seedling growth of *P. gerardiana*.

Acknowledgement

The author is thankful to the Director FRI Deemed to be University.

References

1. Urooj R, Jabeen A. Present status of *Pinus gerardiana* Wall. in Pakistan: A review. Middle East Journal of Business. 2015; 10(4):46-48.
2. Singh NB. Propagation, selection and establishment of clonal seed orchard of chilgoza pine (*Pinus gerardiana* WALL.). The Indian Forester. 1992; 118(12):901-908.
3. Sharma P, Sehgal RN, Anup R. Natural regeneration of *Pinus gerardiana* in dry temperate forests of Kinnaur (Himachal Pradesh). Indian Journal of Forestry. 2010; 33(4):511-518.
4. Harmon ME, Franklin JF, Swanson FJ, Sollins P, Gregory SV, Lattin JD *et al*. Ecology of coarse woody debris in temperate ecosystems. Advances in Ecological Research. 1986; 15:133-302.
5. Bilby RE, Bisson PA. Function and distribution of large woody debris. In: Naiman RJ, Bilby RE (eds) River ecology and management: lessons from the Pacific coastal ecoregion. Springer-Verlag, New York, 1998, 324-346.
6. Akbar M, Khan H, Hussain A, Hyder S, Begum F *et al.* Present status and future trend of chilgoza forest in Gharabad, district Diamer, Gilgit-Baltistan, Pakistan. Journal of Biodiversity and Environmental Sciences. 2014; 5(5):253-261.
7. Yadav RR. Tree rings imprints of long-term changes in Western Himalayas. Indian Journal of Biosciences. 2009; 34:699-707.
8. Sarkar R. Accessibility: A boon or a dilemma: weighing outcomes in an ecologically fragile belt of the middle Himalayas. Proceeding of International Conference on Transportation Systems, Central Building Research Institute, Roorkee, India, February, 2008, 10-12.
9. Czabator FJ. Germination value: an index combining speed and completeness of pine seed germination. Forest Science. 1962; 8:386-396.
10. Abdul-Baki A, Anderson JJ. Vigour determination in soyabean by multiple criteria. Crop Science. 1973; 13:630-633.
11. Kumar R, Shamet GS, Mehta H, Alam NM, Tomar JMS *et al*. Influence of gibberellic acid and temperature on seed germination in Chilgoza pine (*Pinus gerardiana* Wall.) Indian Journal of Plant Physiology. 2014; 19(4):363-367.
12. Cetinbas M, Koyuncu F. Improving germination responses of three congeneric cactus germination of *Prunus avium* L. seeds by gibberellic acid, potassium nitrate and thiourea rarity. Horticultural Science. 2006; 3:119-123.
13. Chen SY, Kuo SR, Chien CT. Roles of gibberellins and abscisic acid in dormancy and germination of red bayberry (*Myrica rubra*) seeds. Tree Physiology. 2008; 28(9):1431-1439.
14. Dhoran VS, Gudadhe SP. Effect of plant growth regulators on seed germination and seedling vigour in *Asparagus sprengeri*. International Research Journal of Biological Sciences. 2012; 1(7):6-10.
15. Davis AS, Jacobs DF. Quantifying root system quality of nursery seedlings and relationship to outplanting performance. New Forests. 2005; 30:235-251.
16. Saeed M, Thanos CA. The effect of seed coat removal on seed germination of *P. gerardiana* Wallich ex D. Don. Chilgoza pine. Journal of Applied and Emerging Sciences. 2006; 1(3):174-177.