UNDER PEER REVIEW

Original Research Paper

Determination of effect of stratification temperature and Duration on germination of Spruce (Picea smithiana Wall. Boiss) under laboratory conditions

Abstract

Aim: The natural regeneration of Picea smithiana is generally slow and almost negligible due to a number of factors e.g. presence of undecomposed raw humus on forest floor, low germinative capacity of seed and infrequent good seed years, therefore the present investigation was carried out to find the effect of different stratification temperatures and durations on germinability.

Area of study: The Seed was collected Anantnag, Jammu and Kashmir, the germination tests were carried out in laboratory at Faculty of Forestry, Faculty of forestry, Sher-e-Kashmir University of Agriculture Science and Technology of Kashmir, Benhama, Ganderbal, Jammu and Kashmir during 2010-2011.

Methodology: For germination tests the seeds were subjected to different stratification temperatures i.e. 0±1OC, 4±1OC, ambient temperature (room temperature) and durations i.e. (15, 30, 45, 60, 75, 90, 105 and 120 days) on germinability of spruce (Picea smithiana Wall. Boiss) seeds so that this important species can be propagated on commercially to avoid it disappearance from the great Himalayas.

Results: The experiment consisted of twenty-four treatments laid out in completely randomized design under laboratory conditions. It was observed that stratification temperature 0±1^{O}C (T_1) for a period of 75 days (P_5) resulted in higher germination of 60.62 % and germination capacity of 69.62 %, which declined to 46.37 % and 56.37 % at the end of 120 days stratification period. Similar trend was observed for the other germination parameters.

Conclusion: This indicated that *Picea smithiana* seeds do possess inherent dormancy which further deepened with storage. Pre-chilling or cold stratification for 75 days at 0±1^{O}C effectively overcame dormancy.

Key words: *Picea smithiana*, stratification, Germination, Dormancy

1. Introduction

Conifer forests are important source of industrial wood and are open treasures, a rich heritage of temperate and subtropical regions of world. Besides providing large quantities of timber, fuel wood and

UNDER PEER REVIEW

fodder, they are the basic source for procurement of non-wood forest reserves and mineral products and are also useful in preservation of natural animal wealth and sustainable ecosystems. In India, conifers occupy extensive forest areas in the Western Himalayan states of Himachal Pradesh, Jammu & Kashmir and Uttrakhand (25). They play a vital part in maintaining the perennial water system, oxygen flow, conserving and protecting the soil resources, enhancing the beauty of landscape and assuring a soluberent climate. The Himalayas are the home of conifers and constitute one of the most divergent and economically important group of species. The conifer species especially Kail (*Pinus wallichiana*), Deodar (*Cedrus deodara*), Fir (*Abies pindrow*) and Spruce (*Picea smithiana*) are distributed on an altitudinal line one above the other in tiers, in pure or in mixed species combination. These conifer species form extensive forests of economic value in the Himalayas. *Picea smithiana* is among one of the important conifers of J&K (1). This conifer species occurs throughout the Western Himalayas from Afghanistan to Kumaun, chiefly at an elevation of 2,000 to 3,200 m amsl, though occasionally descending lower on northern aspects. Spruce is a tall evergreen tree and due to its gregarious nature, it is not often found pure over large area but more commonly associated with fir or with deodar, blue pine, oak (chiefly *Quercus dilatata*) and occasionally at higher elevations with *Q. semicarpifolia* and other level species such as maple (*Acer tournef*), walnut (*Juglans regia*), bird cherry (*Prunus padus*) and elm (*Ulmus wallichiana*) (28).

Conifer seeds in general have a high degree of dormancy even under favourable environmental conditions (12). This dormancy results from a combination of internal (physiological) and external (physical) factors (4). Hard seed coat acts as a barrier for the imbibition of water and exchange of gases (essential for initiation of germination process). Hard seed coats together with pericarps and other structural barriers impose a high mechanical resistance and block water uptake and/or oxygen diffusion (14). Cold stratification has been widely used as a pre-sowing treatment is for enhancing the rate and percentage of germination and for breaking the dormancy (9; 4). This is effortless, cheap and successful method for overcoming the seed dormancy. The effects of moist chilling in establishing hormonal levels have been proved due to initiation of appropriate enzyme activity (21). Moreover, the phenomenon of cold stratification has long been recognized in overcoming physiological dormancy of seeds of many species. Moist chilling breaks the dormancy and accelerates the rate of germination in physiologically dormant *Picea glauca* seeds (18). Moist chilling of 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 (18).

The natural regeneration of spruce (*Picea smithiana*) is generally negligible because of low germination capacity of seed and infrequent seed formation years. Keeping in view the difficulties in getting natural regeneration and the role of chilling duration and incubation temperature in enhancing the germination and other germination related parameters, the present investigation was under taken to study

UNDER PEER REVIEW

the effect of different stratification temperature and durations on germination of this important species under laboratory conditions.

2. Materials and methods

2.1 Seed source

Seed was collected in the form of mature cones from randomly selected trees from the forest area of Daksum (Anantnag) Jammu and Kashmir, India during the seed collection season in both the years (2011 and 2012) separately. The forest area is around 2200 m amsl. Several hundred cones were collected each year and packed in gunny bags for their transportation to the experimental site. The cones were dried under shade at room temperature and the seed was separated from these cones at adequate stage of maturity. The bulk seed, thus collected, was used for research studies. The germination parameters were recorded in laboratory at Faculty of Forestry, Faculty of forestry, Sher-e-Kashmir University of Agriculture Science and Technology of Kashmir, Benhama, Ganderbal, Jammu and Kashmir during 2010-2011.

2.2 Stratification temperatures

During January 2011 and 2012 a large quantity of spruce (*Picea smithiana*) seeds were placed for stratification at three different temperatures in batches separately for each year.

T_1 : Dry cold stratification at 0±1^{O}C

T_2 : Dry cold stratification at 4±1^{O}C

T_3 : No stratification (at ambient temperature-control)

2.3 Stratification Durations

In each year, eight samples of seeds of spruce (Picea smithiana) placed for stratification at different temperatures were drawn separately for each treatment at fifteen days interval. Seeds drawn from the lot placed at ambient temperature worked as a control for comparison. Seeds were afterwards put to germination test.

Duration		Days	Duration		Days
P1	:	15 days	P5	:	75 days
P2	:	30 days	P6	:	90 days
P3	:	45 days	P7	:	105 days
P4	:	60 days	P8	:	120 days

The experiment thus, comprised 24 treatments each replicated 4 times (100 seeds/replication/treatment) and were evaluated in Completely Randomized Design under laboratory conditions. The seeds, after counting, were placed in a Petri dish with two fold germination paper and were placed in a germinator with a calibrated temperature of 20±1OC. Daily observations were recorded up to 28 days for following observations.

UNDER PEER REVIEW

Germination (per cent), germination capacity (per cent), germination energy (per cent), germination speed and germination value.

3. Result and Discussion

3.1 Stratification of seed

Seed dormancy is natural way of setting time clock that allows seed to initiate germination when conditions are favourable for germination and seedling survival (30;32). Embryos described as underdeveloped may be dormant at maturity, thus seeds may need warm and/or cold stratification for embryo growth and germination to occur (21; 4).Various physiological changes occur during the stratification as indicated by increasing/ decreasing levels of total sugars and starch in seeds. Combination of low temperature and stratification duration appear to trigger off biochemical changes to transform complex food substances into simple forms that are used at germination time (17). Cooler temperature during stratification period improves germinability in conifers (5**)**. The high germinability may be due to conversion of starch into monosaccarides and starch that are used for embryo growth and development (17;13).

In the present investigation the seeds collected from the target forest area, in the form of cones, were subjected to stratification temperatures of 0±1^{O}C and 4±1^{O}C under laboratory conditions with room temperature. This stratification was carried over for 4 months (120 days) with seed lots from each stratification temperature taken at regular intervals of 15 days (i.e. 15, 30, 45, 60, 75, 90, 105 and 120 days). These seed lots stratified at different period intervals were put for germinability tests (germination per cent, germination capacity per cent, germination energy per cent, germination speed and germination value) under laboratory conditions. The salient findings are discussed briefly as under:

3.2 Germination per cent

Significant increase in germination (53.81%) was observed as the stratification temperature was lowered to 0±1OC as compared to 50.50 and 46.12 per cent recorded in 4±1OC and control, respectively (Table-1). A linear increase in the per cent germination was recorded up to 75 days stratification, where after it decreased linearly. Maximum germination of 57.29 per cent was recorded in seeds stratified for 75 days, which was significantly higher than 15 and 30 days period (45.62 and 45.70%, respectively). A linear decrease from the maximum germination was observed after 75 days being 57.20 per cent (90 days), 51.04 per cent (105 days) and 44.37 per cent (120 days), the decrease being significant only in seeds stratified for 105 and 120 days. Interaction between the stratification temperature and period revealed that irrespective of a particular temperature, the maximum germination was observed in the seeds stratified for 75 days. A linear increase in germination was observed as the stratification increased up to 75 days where after a linear decrease was observed. The maximum germination of 60.62 per cent was recorded in the seeds stratified for 75 days at 0±1OC followed by 60.37 per cent in the seeds stratified for 90 days at 0±1OC and 59.37 per cent in seeds stratified for 60 days at the same temperature and 75 days at 4±1OC.

UNDER PEER REVIEW

3.3 Germination capacity

Germination capacity measures actual seed germination and potential of non-germinated seeds to germinate after a given time. This gives an index of maturity and viability of seeds. Maximum germination capacity of 63.04 per cent was recorded in the seed lot stratified at 0±1OC which was significantly higher than 61.28 and 56.81 per cent in the seed lots stratified at 4±1OC and ambient room temperature, respectively (Table-2). The highest germination capacity of 67.00 per cent was recorded in the seeds stratified for 75 and 90 days. The effect of interaction of stratification temperature and duration revealed that maximum germination capacity of 69.62 per cent was recorded in seeds stratified for 75 days at 0±1OC, which was closely but non-significantly followed by 69.26 per cent in seeds stratified at the same temperature for 90 days, 69.00 per cent in seed lots stratified for 75 days at 4±1OC and 68.62 per cent in seed lots stratified for 60 days at 0±1OC.

3.4 Germination energy

The seed lots scored for germination per cent and germination capacity after stratification for different periods at different temperature regimes were also having maximum germination energy (Table-3).Germination energy increased linearly and significantly from 23.29 per cent in the control to 26.63 and 29.18 per cent at 4±1OC and 0±1OC, respectively. Maximum germination energy (33.46%) was recorded for the seeds stratified for 75 days, which was significantly higher as compared to all other seeds stratified at different intervals stratification. The minimum value of 22.12 per cent was recorded in the seeds stratified for 15 days followed by 22.16 per cent at 120 days, which differed significantly. The interaction between stratification temperature and duration varied significantly with the maximum germination energy of 35.89 per cent recorded for the seeds stratified for 75 days at 0±1OC followed by the seeds stratified for 75 days at 0±4OC with 33.65%.

3.5 Germination speed

Germination speed measures the germination potential of seeds at a particular time scale and gives an indication of the internal physiological factors inside the seed that enhance and promote the germination process when external factors favorable for germination are kept at optimum level. The highest germination of 9.25 was recorded for the seeds stratified at 0±1OC, which was significantly higher than 8.23 and 7.58 recorded in the seed lots stratified at 4±1OC and ambient room temperature, respectively (Table-4). Stratification periods revealed a linear increase in the germination speed up to 75 days interval, where after it decreased linearly. The highest germination value of 9.55 was recorded in the seed lot drawn on 75th day, followed by 9.33 on 60th day.

3.6 *Germination value*

Maximum value of 6.30 was recorded in the seeds stratified at 0±1OC, which was significantly higher than 4.20 and 3.81 recorded at 4±1OC and ambient room temperature, respectively (Table-5). The difference between the values 3.81 and 4.20 was non-significant. Maximum germination value of 4.53

UNDER PEER REVIEW

was recorded in the seeds lot drawn on 75th day of stratification, which was however, non-significantly different from the values of 4.01, 4.26, 4.44 and 4.46 recorded in the seed lots drawn on 45th, 105th, 60th and 90th day, respectively. The maximum value was however, significantly higher than those recorded on 15th day (3.34), 30th day (3.70) and 120th day (3.75). Considering the effect of interaction of stratification temperature and duration, it was observed that maximum germination value of 4.94 was recorded in the seeds stratified for 75th day at 0±1OC or 4±1OC. Germination values of 3.78 or above at 0±1OC were significantly higher than that recorded on 15th day (2.49).

Moist stratification at low temperatures or pre-sowing in cold water for a period of few hours to several days is reported to increase germination without influencing germination capacity in seeds of spruce (10). After 3 weeks of cold chilling of spruce seeds, a significant increase by 56.00 per cent was recorded in germination over control. Germination value and germination speed recorded a significant increase by 29.70 and decrease by 40.0 per cent, respectively in spruce seeds after 4 weeks of moist chilling (19). Jinks and Jones (11) reported increase in germination speed in *Picea sitchensis* after chilling. Lavania *et al.,* (15) observed an increase in germination by 19.75 per cent after 60 days of stratification of spruce seeds as compared to control. Mugloo et al., (20) reported significant increase in seed germination of *Cryptomeria japonica* up to 45 days of stratification where after it decreased significantly. The cold or winter stratification outdoors successfully overcame seed dormancy for commercial propagation of the trees species (33;6).

Thapiliyal and Gupta (27) reported that germination per cent varied from 16 to 69% in of *C. deodara* seeds and Rawat and Bakshi, (34) also documented wide range of variation with respect to stratification temperature and duration in *Pinus wallichiana*. Sofi and Bhardwaj (26) reported significant improvement in seed germinability parameters of Cedrus deodara after cold moist stratification at 2-3OC for 75 days followed by 60 days of pre-chilling. Malik and Shamet (16) reported significant improvement in seed germination of *Pinus gerardiana* after 60 days of stratification at 4±1OC. Faster germinating seeds are reported to produce more uniform and vigorous seedling that promote the seedling growth and development parameters (23). Stratification is the most common method employed to break the dormancy so as to ensure uniform and quick germination of seeds in many species (8). Gosling (7) postulated that stratification is interpreted as important tool to encourage germinability of dormant seeds that require pretreatment by duration and temperature. It has synchronize germination of most gymnosperm seeds, even those that exhibit no dormancy. Embryo growth occurs at relatively high temperatures (15 0C) in simple morphophysiological dormancy; whereas, in complex morpho-physiological dormancy, embryo growth occurs only at low temperatures (0–10 0C) (2;29).

UNDER PEER REVIEW

Table 1: Effect of different stratification temperatures, periods and their interaction (T x P) on germination per cent of Spruce (*Picea smithiana,* Wall. Boiss) seeds under laboratory conditions pooled over the years 2011 and 2012

Periods / Temperatures	Germination per cent								
	P_1 (15 days)	P_2 (30 days)	P_3 (45 days)	P_4 (60 days)	P_5 (75 days)	P_6 (90 days)	P_7 (105 days)	P_8 (120 days)	Mean
T_1: 0±1^{O}C	49.12	51.62	55.12	59.37	**60.62**	60.37	47.87	46.37	**53.81**
T_2: 4±1^{O}C	46.62	47.12	50.62	55.12	59.37	53.87	46.87	44.37	**50.50**
T_3: Ambient (room temp)	38.37	41.12	42.37	46.62	56.12	53.12	51.37	39.87	**46.12**
Mean	**45.62**	**45.70**	**49.37**	**50.54**	**57.29**	**57.20**	**51.04**	**44.37**	

CD ($p \leq 0.05$)

Temperature (T) = 1.68

Period (P) = 2.75

T x P = 4.78

Table 2: Effect of different stratification temperatures, duration and their interaction (T x P) on germination capacity of Spruce (*Picea smithiana,* Wall. Boiss) seeds under laboratory conditions pooled over the years 2011 and 2012

Periods / Temperatures	Germination capacity (%)								
	P_1 (15 days)	P_2 (30 days)	P_3 (45 days)	P_4 (60 days)	P_5 (75 days)	P_6 (90 days)	P_7 (105 days)	P_8 (120 days)	Mean
T_1: 0±1^{O}C	58.25	59.62	60.37	68.62	**69.62**	69.26	57.87	56.37	**63.04**
T_2: 4±1^{O}C	56.37	56.87	60.37	61.00	69.00	65.25	65.25	64.12	**61.28**
T_3: Ambient (room temp)	47.87	53.16	53.26	56.62	66.12	63.37	56.75	53.87	**56.81**
Mean	**54.79**	**56.16**	**59.45**	**62.41**	**67.00**	**67.00**	**59.58**	**55.50**	

CD ($p \leq 0.05$)

Temperature (T) = 1.23

Period (P) = 2.01

T x P = 3.48

Table 3: Effect of different stratification temperatures, duration and their interaction (T x P) on germination energy of Spruce (*Picea smithiana,* Wall. Boiss) seeds under laboratory conditions pooled over the years 2011 and 2012

Periods / Temperatures	Germination energy (%)								
	P_1 (15 days)	P_2 (30 days)	P_3 (45 days)	P_4 (60 days)	P_5 (75 days)	P_6 (90 days)	P_7 (105 days)	P_8 (120 days)	Mean
T_1: 0±1^{O}C)	**19.13**	25.21	30.49	32.02	**35.89**	33.03	25.07	24.59	**29.18**
	(4.48)	(5.091)	(5.59)	(5.72)	(6.07)	(5.83)	(5.08)	(4.95)	**(5.40)**
T_2: 4±1^{O}C	20.07	26.06	27.00	29.71	35.65	32.36	21.67	20.26	**26.63**
	(4.58)	(5.18)	(5.27)	(5.51)	(5.88)	(5.77)	(4.74)	(4.61)	**(5.22)**
T_3: Ambient (room temp)	20.24	21.87	24.38	30.83	31.42	27.83	25.59	20.20	**23.29**
	(4.56)	(4.76)	(5.00)	(5.63)	(5.68)	(5.36)	(5.14)	(4.60)	**(4.82)**

Mean	**22.12 (4.77)**	**26.97 (5.19)**	**27.87 (5.27)**	**28.17 (5.30)**	**33.46 (5.78)**	**27.67 (5.33)**	**24.43 (4.94)**	**22.16 (4.70)**	

CD ($p \leq 0.05$)

Temperature (T) = 0.21
Period (P) = 0.37
T x P = 0.64

Table 4: Effect of different stratification temperatures, duration and their interaction (T x P) on germination speed of Spruce (*Picea smithiana,* Wall. Boiss) seeds under laboratory conditions pooled over the years 2011 and 2012

Periods / Temperatures	Germination speed								
	P_1 (15 days)	**P_2 (30 days)**	**P_3 (45 days)**	**P_4 (60 days)**	**P_5 (75 days)**	**P_6 (90 days)**	**P_7 (105 days)**	**P_8 (120 days)**	**Mean**
T_1: 0±1^{O}C)	5.43	6.60	7.61	9.33	**10.92**	9.04	7.97	5.86	**9.25**
T_2: 4±1^{O}C	6.86	7.88	8.06	8.94	9.53	9.30	7.43	6.78	**8.23**
T_3: Ambient (room temp)	6.82	8.78	9.28	10.37	10.59	10.18	9.34	8.70	**7.58**
Mean	**6.37**	**8.42**	**8.68**	**9.33**	**9.55**	**8.81**	**8.11**	**7.54**	

CD ($p \leq 0.05$)

Temperature (T) = 0.80
Period (P) = 1.31
T x P = 2.28

Table 5: Effect of different stratification temperatures, duration and their interaction (T x P) on germination value of Spruce (*Picea smithiana* Wall. Boiss) seeds under laboratory conditions pooled over the years 2011 and 2012

Periods / Temperatures	Germination value								
	P_1 (15 days)	**P_2 (30 days)**	**P_3 (45 days)**	**P_4 (60 days)**	**P_5 (75 days)**	**P_6 (90 days)**	**P_7 (105 days)**	**P_8 (120 days)**	**Mean**
T_1: 0±1^{O}C)	2.49	3.78	4.26	4.89	**4.94**	4.67	4.64	3.78	**6.30**
T_2: 4±1^{O}C	3.68	3.70	4.06	4.65	4.94	4.44	4.04	4.08	**4.20**
T_3: Ambient (room temp)	2.54	3.35	3.35	4.67	4.67	4.15	3.46	3.31	**3.81**
Mean	**3.34**	**3.70**	**4.01**	**4.44**	**4.53**	**4.46**	**4.26**	**3.75**	

CD ($p \leq 0.05$)

Temperature (T) = 0.86
Period (P) = 0.58
T x P = 1.01

UNDER PEER REVIEW

UNDER PEER REVIEW

4. Conclusion

The study confirms that seeds of Picea smithiana have linear underdeveloped embryos at maturity, and exhibit intermediate complex morpho-physiological dormancy. Pre-chilling or cold stratification for 75 days at 0±1OC effectively overcame dormancy, and accelerated seed germination of Picea smithiana and could be used for commercial propagation. Embryonic growth and development occurred during chilling at 0±1OC.

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UNDER PEER REVIEW

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UNDER PEER REVIEW

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