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Effect of Different Pre-Sowing Treatments on Seed Germination of Spruce (*Picea smithiana* Wall. Boiss) Seeds under Temperate Conditions of Kashmir Himalayas, India

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A B S T R A C T

The present study was carried out in laboratory at Faculty of Forestry Sher e-Kashmir University of Agriculture Science and Technology of Kashmir, Benhama, Ganderbal, Jammu and Kashmir during 2011-2012 to investigate the effect of different GA_3 concentrations *viz.* 50, 100, 150, 200 and 250 ppm on germinabiliy and growth of seeds *Picea smithiana* under laboratory and nursery conditions imbibed for 0, 24 and 48 hours durations. From this study it was found that the seeds treated with 200 ppm GA_3 for 48 hours produced better germinability and growth both under laboratory and nursery conditions. The maximum germination percentage under laboratory recorded was 70.62 % with the germination capacity of 80.75 %, germination energy of 48.99, germination speed of 28.52 and germination value of 5.90.Whereas the maximum germinability *viz.*, germination percentage, germination, germination value and plant percent of 64.00, 34.21, 2.82 and 54.50 respectively. The germination results indicated that *Picea smithiana* seeds do possess inherent dormancy which increases with storage. Therefore it is advised that spruce seeds should be imbibed in 200 ppm GA_3 for 48 hours for better germination and growth.

Introduction

Conifer seeds in general have a high degree of dormancy even if subjected to environmental conditions favourable for germination (Jull and Blazich, 2000). This dormancy is caused by a combination of internal (physiological) and external (physical) factors (Basu, 1994). Hard seed coat acts as a barrier for the imbibition of water and exchange of gases, essential for initiation of the 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 (Kelly, 1992). Cold stratification has been widely used as a pre-sowing treatment for breaking dormancy to enhancing the seed germination rate (ISTA, 1976; Baskin and Baskin, 2004). This is an effortless, cheap and successful method for overcoming seed dormancy. The effects of moist chilling in establishing hormonal levels have been proved due to initiation of appropriate enzyme activity (Nikolaeva, 1969). Moreover, the phenomenon of cold stratification has long been recognized in

overcoming physiological dormancy of seeds of many species (Baskin, and Baskin, 1987). Moist chilling breaks the dormancy and accelerates the rate of germination in physiologically dormant *Picea glauca* seeds (Wang, 1987). 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 (Mittal, 1987).

Materials and Methods

The present study "Effect of different pre-sowing treatments on seed germination of spruce (*Picea smithiana* Wall. Boiss) seeds under temperate conditions of Kashmir Himalayas" was carried out in laboratory at Faculty of Forestry Sher e-Kashmir University of Agriculture Science and Technology of Kashmir, Benhama, Ganderbal, Jammu and Kashmir during 2011-2012 to investigate the effect of different GA_3 concentrations on germinabiliy and growth of seeds *Picea smithiana* under laboratory and nursery conditions which were imbibed for three different durations. The treatment details are given in table below:

The experiment comprised of 13 treatments combinations (100 seeds/replication) in completely randomized design under laboratory conditions. After counting, seeds were placed in a petri dish with two fold germination paper and placed in a germinator with a calibrated temperature of 25±1°C. All treatments were examined daily, seeds were considered germinated when the radicle was 5 mm long. Germination percentage and germination capacity, germination energy, germination speed and germination value was recorded daily following formulas given by (Sosa, 2005) as bellow:

$$\text{Germination percentage (GP)} = \left(\frac{n}{N}\right) \times 100$$

$$\text{Germination capacity (GC)} = \left[\frac{(v+n)}{N}\right] \times 100$$

where, n is the number of germinated seeds, N is the total number of seeds, v is the number of viable seeds recorded after conducting viability test using tetrazolium chloride (Peters, 2000) and D is the number of days to final germination.

Germination energy, germination speed and germination value was determined using the following formula given by Czabator (Czabator, 1962):

$$\text{Germination energy (GE)} = \left(\frac{M}{N}\right) \times 100$$

Where, M is cumulative germination up to time of maximum MDG reached at any time during the period of the test, N is the total number of seeds, N is the total number of seeds,

$$\text{Germination speed (GS)} = \sum\left(\frac{n}{d}\right)$$

Where, n = number of germinated seeds, d = number of days

Germination value (GV) = PV × MDG

Where, PV is the peak value of maximum means daily germination reached at any time during the period of the test.

The treated seeds were simultaneously sown in poly-bag (4″x7″) also during the first week of the February, 2011 and 2012 in nursery. For the following paramaters: Germination (%), Germination energy (%), Germination value, Seedling height (cm), Collar diameter (mm), Root/shoot weight (g), Root:shoot ratio, Total biomass (g), Plant per cent.

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) (5 %) was calculated as: CD = SEd X $t_{0.01}$, Where, SEd is the standard error of difference calculated as SEd = √2Me/r, where Me= mean sum of square and r= number of replicates.

Results and Discussion

Enhancement of seed germination and improvement in seedling growth is controlled by plant hormones under favourable growth conditions. Several growth hormones are associated with seed germination and seedling physiology, but the most important being gibberellins, IAA and Kinetin (Faridi *et al.*, 2000). These growth hormones function via activation of enzymes, mobilization of food materials leading to cell division, cell elongation and embryo growth that promotes germination in viable seeds (Khan, 1980). During germination and growth of embryonic axis, the carbohydrates and sugars are utilized to meet the requirement of active respiration and synthesis of cell wall and protoplasm materials for dividing and growing cells. The end products are transported to the growing axis to again provide raw materials for early growth of very young seedlings (Black, 1992). In the present investigation good quality spruce seeds were soaked in water for 24 or 48 h and also given GA_3 treatment at 50, 100, 150, 200 or 250 ppm. Control seeds were not soaked or applied GA_3. A total of 13 treatment combinations were evaluated for their impact on seed germinability parameters under laboratory and field conditions and seedling growth parameters under field conditions.

Seed germination parameters

Laboratory conditions

Gibberellins are the naturally occurring plant growth hormones. GA_3 treatment can overcome dormancy in different seeds that have hard seed coat or dormant embryo. In most of the species survival percentage, growth and total biomass increased when seeds are pretreated with GA_3. The result of the influence of gibberellic acid on seed germinability is presented in figure 1. Germination percentages of *Picea smithiana* seeds with or without soaking by in GA_3 over varied periods differed significantly ($p \geq 0.05$).Without GA_3 seeds germinated low and started late. In contrast, when seeds were imbibed in different concentrations of GA_3 for varying durations, the germination percentage rose to 39.50 % without soaking (Control) to 75.50 % when seeds were soaked in 200 ppm GA_3 for 48 hrs, soaking alone in distilled water and lower concentrations of GA_3 was ineffective in breaking dormancy of the seeds fully to produce the maximum germinability of the viable seeds, indicating that *Picea smithiana* seeds have physiological dormancy. Similar trend was observed for the other germination parameters viz. germination capacity (85.50 %), germination energy (55.46), germination speed (32.89) and germination value (10.58) when the seeds were imbibed in 200 ppm GA_3 for 48 hrs and differed significantly from the seeds which were socked 24 hour in 200 ppm GA_3 with germination percentage of 70.62 %, germination capacity of 80.75 %, germination energy of 48.99, germination speed of 28.52 and germination value of 5.90. The minimum germination parameters were recorded in the seeds which were sown without any treatment (control). The germination parameters increased with the increase in the GA_3 concentration and socking duration up to 200 ppm and socked for 48 hours and decreased with the further increase in the GA_3 concentration and socking duration.

Nursery conditions

Germination of the seeds treated with different GA_3 concentrations (Fig. 2) revealed

that germination parameters viz. germination percentage and germination energy increased and varied significantly at from p ≤0.05 from 30.11 % and 10.58 % (control) to 64.00 to 34.21 % (200 ppm GA_3 for 24 hours) respectively. Similarly germination value and plant percent increased linearly and significantly at p ≤0.05 from 0.75, and 19.90 % under control and reached maximum with 2.82 and 54.50 respectively when the seeds were treated with 200 ppm GA_3 for 24 hours (Table 1) and decreased gradually after the further increased in the GA_3 concentration and duration of imbibition under field conditions.

Application of GA_3 exogenously has been reported to confer many beneficial effects. Omran *et al.,* (1980) reported that 25 ppm GA_3 increased germination by about 40 per cent in zeera seeds. Also found an increase by 39.0 per cent in the seeds of *Hibiscus esculenta* at 400 ppm GA_3 application. Increase in spruce of seed germination by optimum concentration of GA_3 might probably have been due to enhancement of hydrolase (especially δ-amlyse) synthesis as reported by Paleg (1960a and b; Amen 1968 or probably due to first initiation of embryo growth and subsequent synthesis of more GA_3 that might have induced hydrolase synthesis (Chen and Varnes, 1973). Several studies have shown gibberellins to be an effective germination stimulator (Sofi, 2005; Lavania *et al.,* 2006). An increase in germination of chilgoza pine seeds by increasing soaking periods was probably attributed to enhancement of hydrolase (especially amylase) synthesis, as reported by Bewley and Black (1994) and Chen *et al.,* (2008)

ABA is reported to be present in the pericarp and seed coat of some plant species that inhibits germination (Leadem, 1987). Cold stratification followed by GA_3 application has been found to suppress this inhibition and enhance germination (Mcbridge and Dickson, 1972) spruce seeds. Shivani (2003) observed increase in the germinability of *Abies pindrow* seeds after 24 h soaking in water at 2-3^{O}C under laboratory conditions and 48 h water soaking at 2-3^{O}C under field conditions, followed by 200 ppm GA_3, soaking for 24 h.

In *Picea smithiana* soaking of seeds for 24 h at 2-3^{O}C + 100 ppm GA_3 application increased germination under laboratory conditions but under field conditions 48 h soaking in water was good. Lavania *et al.,* (2006) observed that for higher germination *Pinus wallichiana* seeds required more soaking period for lower GA_3 concentration (100 ppm for 36 h) than for higher concentration (200 ppm GA_3 for 24 h) to get the comparable germination.

Exogenous application of GA3 has been reported to be effective in breaking dormancy and substituting for the chilling requirement in seeds of many species (Smiris *et al.,* 2006; Pipinis *et al.,* 2012).

The treatment details are given in table below

S. no	Treatment details	S. no	Treatment details
T_1	Control	T_8	Soaking in GA_3 (150ppm) for 24 hours
T_2	Soaking in cold water for 24 hours	T_9	Soaking in GA_3 (150ppm) for 48 hours
T_3	Soaking in cold water for 48 hours	T_{10}	Soaking in GA_3 (200ppm) for 24 hours
T_4	Soaking in GA_3 (50ppm) for 24 hours	T_{11}	Soaking in GA_3 (200ppm) for 48 hours
T_5	Soaking in GA_3 (50ppm) for 48 hours	T_{12}	Soaking in GA_3 (250ppm) for 24 hours
T_6	Soaking in GA_3 (100ppm) for 24 hours	T_{13}	Soaking in GA_3 (250ppm) for 48 hours
T_7	Soaking in GA_3 (100ppm) for 48 hours		

Fig.1 Effect of pre-sowing treatments on seed germinability of Spruce (*Picea smithiana*, Wall. Boiss) seed under labortary conditions pooled over the year 2011 and 2012

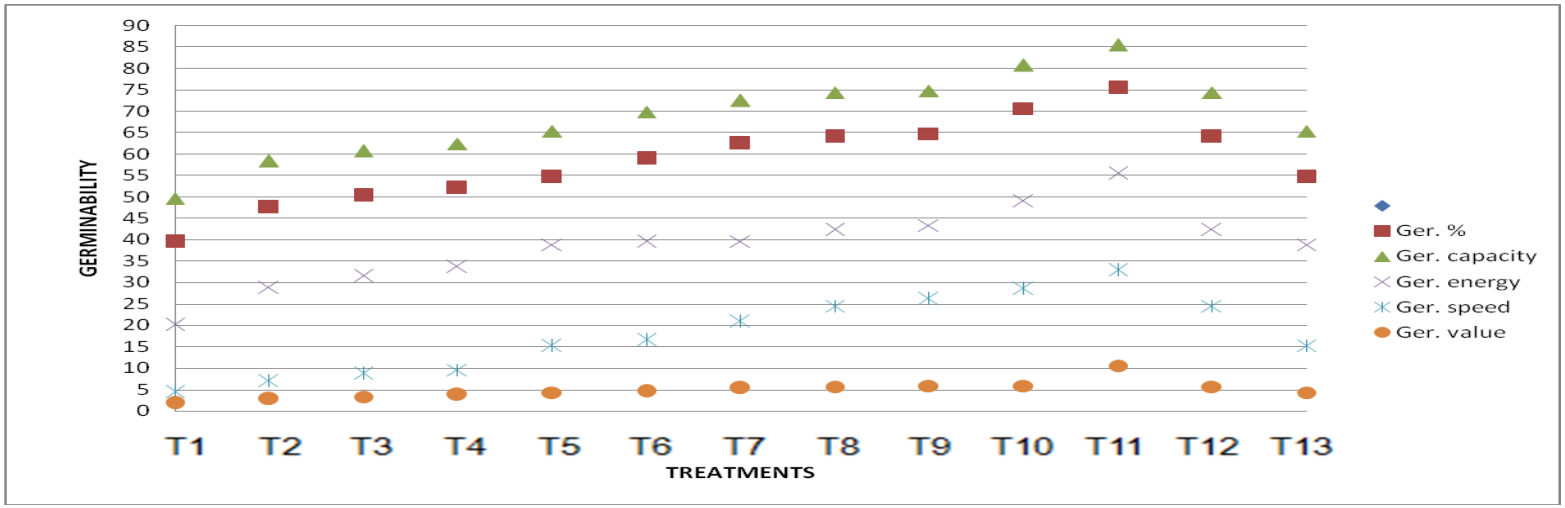

Fig.2 Effect of pre-sowing treatments on seed germination (%)and germination energy of Spruce (*Picea smithiana*, Wall. Boiss) seed under field conditions pooled over the year 2011 and 2012

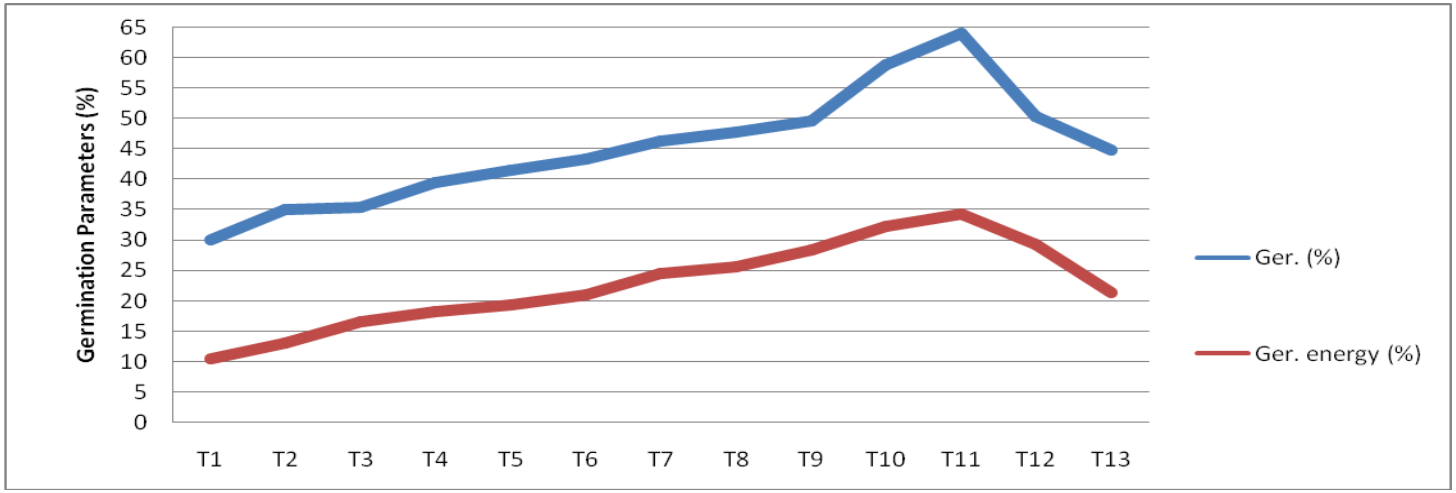

Table.1 Effect of pre-sowing treatments on seed germination value and plant per cent of Spruce (*Picea smithiana*, Wall. Boiss) seed under field conditions pooled over the year 2011 and 2012

Treatments	Germination value	Plant per cent
T_1: Control	0.75	19.90 (4.40)
T_2: Soaking in cold water for 24 hrs	0.88	23.33 (4.90)
T_3: Soaking in cold water for 48 hrs	1.00	25.00 (5.00)
T_4: Soaking in GA_3 50 ppm for 24 hrs	1.05	29.83 (5.54)
T_5: Soaking in GA_3 50 ppm for 48 hrs	1.08	31.50 (5.61)
T_6: Soaking in GA_3 100 ppm for 24 hrs	1.21	35.66 (5.97)
T_7: Soaking in GA_3 100 ppm for 48 hrs	1.44	36.83 (6.15)
T_8: Soaking in GA_3 150 ppm for 24 hrs	1.52	38.16 (6.17)
T_9: Soaking in GA_3 150 ppm for 48 hrs	1.74	40.16 (6.33)
T_{10}: Soaking in GA_3 200 ppm for 24 hrs	1.86	49.33 (7.02)
T_{11}: Soaking in GA_3 200 ppm for 48 hrs	**2.82**	**54.50** (7.38)
T_{12}: Soaking in GA_3 250 ppm for 24 hrs	1.89	40.50 (6.36)
T_{13}: Soaking in GA_3 250 ppm for 48 hrs	1.22	34.83 (5.90)
CD ($p \leq 0.05$)	**0.04**	**0.54**

Figures in parentheses are square root transformed values.

Table.2 Effect of pre-sowing treatments on growth and biomass of Spruce (*Picea smithiana*, Wall. Boiss) seedling under field conditions pooled over the year 2011 and 2012

Treatments	Plant height (cm)	Collar diameter (mm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Shoot root ratio	Total biomass (g)
T_1: Control	1.967	0.218	0.083	0.013	0.027	0.013	1.167	0.110
T_2: Soaking in cold water for 24 hrs	2.200	0.302	0.063	0.009	0.010	0.009	1.000	0.073
T_3: Soaking in cold water for 48 hrs	2.967	0.586	0.142	0.022	0.108	0.012	1.833	0.250
T_4: Soaking in GA_3 50 ppm for 24 hrs	3.100	0.671	0.180	0.010	0.130	0.010	1.000	0.310
T_5: Soaking in GA_3 50 ppm for 48 hrs	3.200	0.807	0.210	0.013	0.147	0.030	0.433	0.357
T_6: Soaking in GA_3 100 ppm for 24 hrs	3.333	0.935	0.227	0.068	0.207	0.028	2.428	0.434
T_7: Soaking in GA_3 100 ppm for 48 hrs	3.600	1.167	0.233	0.033	0.150	0.017	1.941	0.383
T_8: Soaking in GA_3 150 ppm for 24 hrs	3.633	1.185	0.260	0.063	0.230	0.033	1.909	0.490
T_9: Soaking in GA_3 150 ppm for 48 hrs	4.833	1.205	0.268	0.062	0.208	0.025	2.480	0.476
T_{10}: Soaking in GA_3 200 ppm for 24 hrs	4.367	1.268	0.268	0.075	0.220	0.030	2.500	0.498
T_{11}: Soaking in GA_3 200 ppm for 48 hrs	**5.000**	**1.330**	**0.293**	**0.085**	**0.233**	**0.033**	**2.578**	**0.53**
T_{12}: Soaking in GA_3 250 ppm for 24 hrs	4.800	1.322	0.290	0.083	0.222	0.029	2.568	0.512
T_{13}: Soaking in GA_3 250 ppm for 48 hrs	2.950	0.666	0.162	0.028	0.123	0.020	1.400	0.285
CD ($p \leq 0.05$)	**0.233**	**0.089**	**0.020**	**0.013**	**0.011**	**0.013**	**1.025**	**0.026**

Ghayyad *et al.*, (2010) reported that GA3 is effective in shortening the chilling requirement. However, in the present study, the application of GA3 treatments separately showed zero germination percentage.

Seedling growth parameters

The results (Table 2) revealed the seedling growth characteristics improved and varied significantly at p≥ 0.05 with the increase in GA_3 and soaking duration maximum seedling growth parameters *viz*. plant height of 5.00 cm, collar diameter with 1.33 mm, fresh shoot weight with 0.29 g, dry shoot weight with 0.08 g, fresh root weight with 0.23 g, dry root weight with 0.03 g, shoot root ratio with 2.5 and total biomass with 0.53 g were recorded when the seeds were soaked in 200 ppm GA_3 for 48 hours and decreased with the further increase GA_3 concentration, which was at par with the seeds which were soaked in the 250 ppm for 24 hours with the plant height of 4.800 cm, collar diameter 1.32 mm, fresh shoot weight 0.29 g, dry shoot weight 0.083 g, fresh root weight 0.22 g, dry root weight 0.029 g, shoot root ratio 2.56 and total biomass 0.512 g. Whereas the minimum seedling growth parameters were recorded in the seeds which were directly sown in the poly bags without any treatment (control).

The increase in growth of the three species studied under high GA_3 level may be due to increased cell elongation, cell division and stem elongation, resulting in an increased plant growth. The findings with pretreatment of seeds are similar to observations of other investigations (Singh *et al.*, 1984) who have reported that GA_3 enhances the growth of seedlings of several forest tree species. Cytokinins does not appear essential for seed germination but during germination, cytokinins appear to offset the effect of inhibitors, notably ABA. It has been described, as playing a permissive role in germination in allowing gibberellins to function (Leubner-Metzger, 2005). Gibberellins prominently involved in seed germination and mobilization of endosperm reserves during early embryo growth as well as flower and fruit development Hopkins and Huner (2004). It was found that GA increases the growth potential of embryo and promotes germination and is necessary to overcome the mechanical restraint conferred by the seed covering layers by weakening of the tissues surrounding the radicle (Finch-Savage and Leubner-Metzger, 2006).

The preceding results suggest that GA_3 exerted a significant influence on growth growth parameters. This influence on growth parameters might be explained through the role of GA_3 in enhancing gibberellin synthesis, which in turn leads to increase in the branching and their overall growth (Penfield *et al.*,2005). The results of the present study are in agreement with Parvin *et al.*, (2015) who reported root length of 24.58 cm, root volume of 7.63 cm^3 and root area of 18.64 cm^2 for *Juglans nigra* subjected to 2 months of stratification and application of 400 ppm GA_3.

GA_3 has been found to stimulate the growth of stems particularly those of rosette plants (Jones, 1973). Soaking of seeds in different concentrations of GA_3, IAA and IBA has been found to increase cell division, cell elongation and chlorophyll synthesis (Mukaila *et al.*, 1997). Pandiya (1989) has reported that GA_3 application hastens seed germination by inducing embryo development and/or neutralizing the growth inhibitors present in the seed coat.

Among the thirteen different pre-sowing treatments, under laboratory conditions, the maximum germination per cent (75.50), germination capacity (85.50%), germination energy (55.46%), germination speed (32.89)

and germination value (10.58) were recorded in seeds treated with GA_3 200 ppm for 48 hours. Under nursery conditions, the maximum germination (64.00%), germination energy (34.21%) and germination value (2.82), plant height (5.00 cm), collar diameter (1.330 mm), fresh shoot weight (0.293 g), dry shoot weight (0.085 g), fresh root weight (0.233 g), dry root weight (0.033 g), shoot root ratio (2.578), total biomass (0.526 g) and plant per cent (54.50%) was recorded in GA_3 200 ppm seeds soaked for 48 hours (T_{11}). Therefore it is recommended that spruce seeds should be imbibed in 200 ppm GA_3 for 48 hours for better germination and growth.

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