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Seed Source Determines Germination and Survivability in *Saussurea obvallata* (DC.) Edgew. an Endemic Herb of Indian Himalayan Region, India

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ABSTRACT

The present study aims to develop conservation protocol for an endangered alpine medicinal herb *Saussurea obvallata* (DC.) Edgew. (Sacred lotus) through seeds and to find out the impact of seed source, seed mass and pre-treatments on germination parameters and seedling survivability. Seeds were collected from two locations of Uttarakhand i.e., Bedni (BN) and Sundardunga (SD), seed mass for each source were recorded and seeds were pre-treated with different pre-sowing treatments, i.e. GA_3 (GA), KNO_3 (KN) and Thiourea (TU) and kept in seed germinator (SG) (Temperature 25.0±2.0 °C and RH 80%) and Greenhouse (Temperature 21.5±3.30 °C and RH 65-80%). Seeds from SD were found 34% longer ($p<0.001$), 22% wider ($p = 0.01$) and 56% heavier ($p<0.001$) followed by higher germination and survivability in comparison to BN seeds. Under Seed germinator and Greenhouse the seeds from SD germinate 2 days earlier than BN seeds followed by higher germination percentage, seedling morphology and biomass. The seedling length and biomass showed significant positive correlation with seed mass. The present study suggests that healthy seeds determine higher germination and survivability, which may beneficial for designing the conservation strategies for endangered plant species.

Key words: Saussurea obvallata *(DC.) Edgew. Germination, Sacred lotus, Alpine, Native, Conservation*

Introduction

About 15% of land surface of the world is covered by alpine regions, and the species from these regions are strongly influenced by climate change (Inouye, 2020) because they are endemic to environmental factors of a specific region (He *et al.*, 2019). The alpine plants are important for ecosystem services and anthropogenic pressure; Changes in land use and global warming pose survival threats on these species (Jalli *et al.*, 2015). The ex-situ conservation of threatened species is widely accepted strategy by International treaties, conservation and legislation authorities, the Convention on Biodiversity Conservation also recommended ex situ conservation of threatened plant species (Müller *et al.*, 2017). To safeguard alpine species by ex- situ conservation approach through seed bank can be a way to habitat restoration by providing seedlings for in-situ conservation (Mondoni *et al.*, 2011). The responses of many plant species to abiotic factors mostly depends on seeds, which enable re-establishment and regeneration of plants (Satyanti *et al.*, 2011). Seed size or mass is an important factor can influence germination and seedling establishment; the seed mass is greatly influenced by environmental factors

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(Kolodziejek, 2017). To sustain in an unpredictable climatic situation alpine plants shows diverse germination responses (Black, 1995). The present study has been carried out to identify the role of seed source and mass on the germination and earlier seedling establishment for conservation and regeneration of *S. obvallata* by providing quality propagating material. The *Saussurea obvallata* (DC.) Edgew. belongs to the alpine largest family Asteraceae. Saussurea is a huge significant genus because most of the species of this genus are well known at the international level. It is an endemic herb of the Himalayan region and found between 3000-4800m asl. Generally known as Brahma Kamal and it is also state flower of Uttarakhand, India (Kamal B, 2013). In western Himalayan region the species is limited above naval zone, due to climate change and increased anthropogenic disturbances at higher alpine regions the conservation strategy for this species is much needed. In Uttarakhand, the flowers of this species are harvested by local people for religious purposes before maturation of seeds is one of the major factors for its declining diversity in Western Himalaya. The plant holds immense sacred value in Uttarakhand and used as traditional, medicinal, ornamental and religious purposes, Because of its inaccessible habitats very few studies has been carried out (Semwal *et al.,* 2019). The plant *S. obvallata* is also used in the preparation of traditional medicines for the treatment of bone ache, intestinal ailment, urinary tract problems and cold/cough (Kirtikar *et al.,* 1984; Negi, 1999; Saklani *et al.,* 2000). In our notice, there are no previous studies on the ex-situ propagation through seeds and further transplantation at natural habitat.

Materials and Methods

Seed Collection

Mature seeds of *S. obvallata* were collected from two different alpine regions, i.e. Sunderdunga, Bageshwar 30°09′01.24"N, 79°51′25.47"E (3551msl) and Bedni (BN), Chamoli 30°12′17.46"N, 79°40′21.99"E (3731 msl) in the month of October. The collected seeds were processed and dried in partially shaded condition and stored in air tight plastic zip bag at 4 °C temperature at laboratory of High altitude plant physiology research centre (HAPPRC), Srinagar Garhwal, Uttarakhand till the seed germination study. The Seed germination study has been carried out by using seed germinator and Greenhouse facility of the HAPPRC as follows.

Recording of seed attributes

Prior to germination experiment dried seeds of the *S. obvallata* were weighed to determine average seed weight, for this purpose, three replicates of seeds, each replicate of 10 seeds for each seed source were weighed and recorded, data used for constructing relation with germination and morphological parameters. Mean seed weight of three lots (10 seed each lot) was 19.27±3.30 mg for BN, which yields average seed weight 1.92 mg per seed, while the seed weight for SD seeds was 30.05±3.58 mg, giving average seed weight 3.00mg. Average seed length 0.46±0.17 cm per seed was recorded for BN, while average seed length 0.62±0.16 cm per seed was recorded for SD. Average seed width 0.75±0.16mm per seed was recorded for BN, while average seed width 0.92±0.30 mm for SD collected seeds. Seed weight was recorded by electronic weighing balance SARTORIUS (CPA224), seed width was recorded by Vernier Digital caliper (YAAYO) and seed length were recorded by measuring scale.

Seed viability test

To ensure that the seeds used for the experiment were viable and of high quality, the sample lots were subjected to viability test using the tetrazolium technique (Peters 2000). Twenty seeds (3 replicates) were subjected to 2,3,5, triphenyl tetrazolium chloride (TTC) test after 1 month of storage at 4 °C. In this method, seeds were longitudinally sectioned and the sections were immersed in a 0.5% aqueous solution of TTC (pH 6.5) for 24 h at room temperature (25 °C) under dark conditions. The TTC solution was drained and sections were rinsed 2-3 times with water. After 24 hrs seeds color turned in pinkish indicates that seeds are viable.

Pretreatments

Seeds were surface sterilized using 0.5% $HgCl_2$ for 2 min, prior to germination experiment. After that seeds were pretreated with different concentrations (5 μM, 10 μM and 15 μM) of $GA_,$ $KN_,$ and TU for 24h and tested against distilled water (control). Pretreated seeds were placed in glass petri dishes containing Whatman number 1 filter paper (90 mm), each treatment replicated thrice by placing 20 seeds in each petri dish, all petri dishes were kept inside seed germinator (SG-11-D, SR Lab, India) at 25°C in temperature and 16h light and 8h dark condition. Similar of pretreated seeds was sown (at 2 cm depth) in Styrofoam trays containing mixture of Soil, Sand, Farm Yard Manure (FYM), and Forest Litter (FL) in the ratio of 1:1:1:1 and kept in Greenhouse condition **(Photoplate 1).** The day temperature under Greenhouse was 21.5±3.30 °C while night temperature was 8.60±2.45 °C and the relative humidity was between 65-80% during the experiment. PGR (Gibberellic acid) and chemicals (Tetrazolium, Potassium Nitrate and Thiourea) were purchased from Himedia and Sigma Aldrich.

Seedling Transplantation and parameters measurements

Germinated seedlings were further transplanted in open beds at Tungnath (3400masl) located between 30°14′ N latitude and 79°13′E longitude in Rudraprayag district of Garhwal, Uttarakhand Himalaya, India, in the month of July. The mean air temperature during the transplantation was 20 °C, The survivability percentage recorded after 30 days of transplantation of seed germinator seeds and after 60 days of germination of greenhouse seeds were recorded. After 60 Days of transplanted the seedling length and weight were recorded.

Data analysis

a. Germination%: Seed germination percentage was calculated using the following formula given by ISTA (1999)

$$\text{Germination \%} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

b. Mean germination Time (MGT)

Mean germination time was calculated by the formula given by Ellis and Roberts (1981).

$$\text{MGT} = \frac{n1 \times d1 + n2 \times d2 + n3 \times d3 + \cdots}{\text{Total number of days}}$$

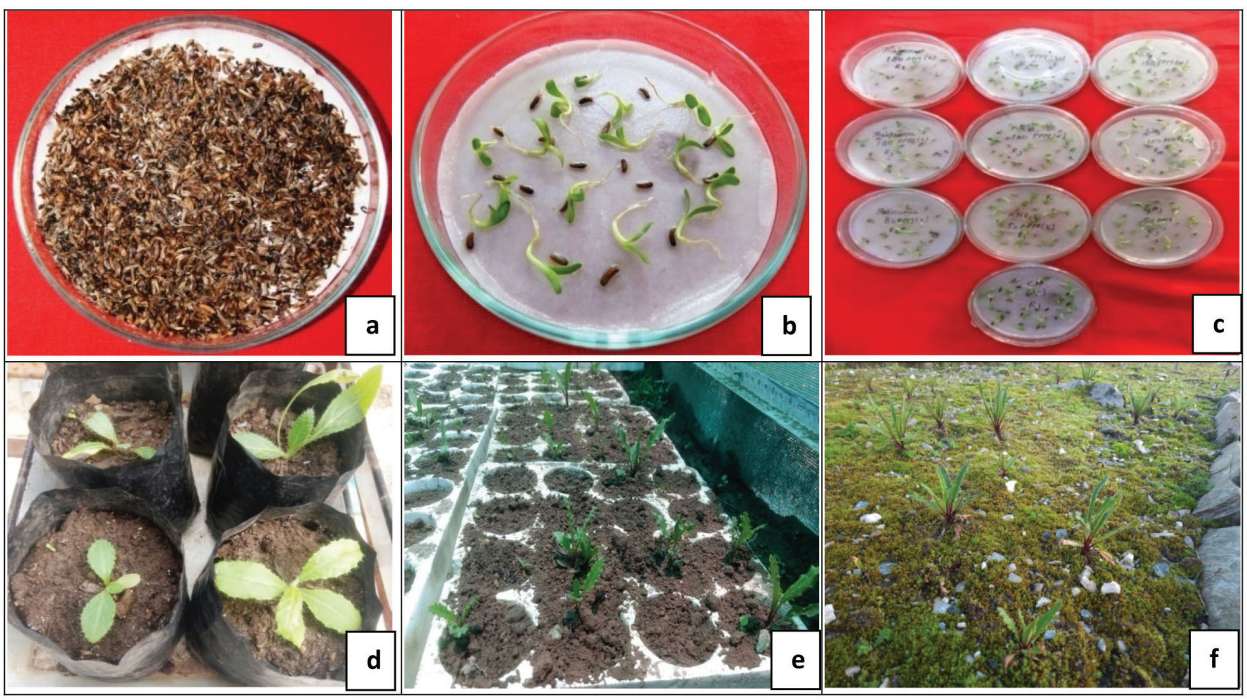
Where, n= number of germinated seed
d = number of days

c. Speed of germination (SG)

Speed of germination was calculated by the following formula given by Czabator (1962).

$$\text{Speed of germination} = \frac{n1}{d1} + \frac{n2}{d2} + \frac{n3}{d3} + \cdots$$

Where, n = number of germinated seeds, d=



Photoplate 1. Germination trails of *Saussurea obvallata* seeds: - Mature seeds of *S. obvallata* (a), seeds sowing in petri plates containing filter paper (b), seed start germination in different pre-treatments in petri plates (c), seedlings in styrofoam trays containing soil mixture (d), seedlings after one month of germination (e) and well grown seedlings of *S. obvallata* after two years (f).

number of days.

d. Survivability%

$$\text{Survivability \%} = \frac{\text{Survived Seedlings after 30 or 60 days}}{\text{Total number of seeds}} \times 100$$

Statistic analysis: The two way analysis of variance (ANOVA) was estimated, source and PGRs treatment were considered as independent factor and their effect on MGT, Germination%, speed of germination were measured. Duncan multiple range test were used to show the difference between PGRs effect for each seed source sown in germinator and greenhouse. General linear model was used to predict the effect of seed mass on germination and seedling establishment. All the analysis was conducted using IBM SPSS (Version-22).

Results

Seed morphology and mass

The student t test showed that the seed morphological parameters were significantly different in SD and BN habitat. The seeds collected from SD were 34.8% (t = 3.57, p =0.001) longer, 22.5% (t = 2.68, p= 0.01) wider and 56% (t = 11.82, p<0.001) heavier in comparison to seed from BN. The seed length and width didn't show any significant effect on germination percentage, but the seed weight had a positive correlation ($r_{(n=60)} = 0.89$, $p<0.001$) with germination percentage. The germination percent was plotted against the seed mass and the regression line explained 73.6% and 54.3% of total variation in SD and BN collected seeds respectively (Figure 1).

Seed viability and Germination percentage (%)

Tetrazolium test resulted in 100% viability of the seeds stored at refrigerator at (4°C). Pretreated seeds sowed in Petri plates and kept inside the seed germinator had started germination after 2 days of

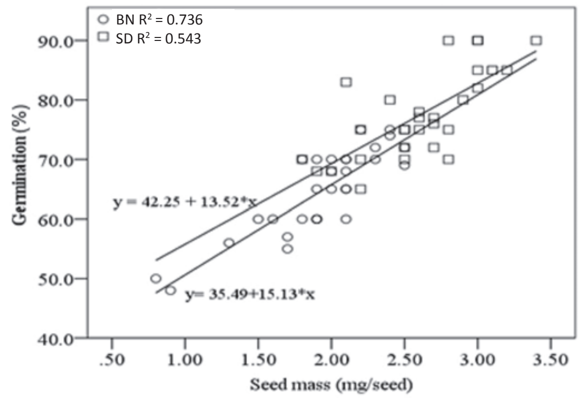


Fig. 1. Relationship between seed germination percentage and seed mass for two seed sources i.e. Round open circle for Vedni (BN) and square for Sundardunga (SD).

sowing, while seeds sowed in soil mixture and kept inside the GH started germination after 11 days of seed sowing. Results from two-way ANOVA showed that the effect of seed source and the interaction between seed source and treatment on germination percentage was found significant (Table 1).

The germination percentage inside seed germinator was found maximum (78.33%) for seed source SD, treated with TU 50 ppm which was about 21.66% higher than control (56.67%), while for BN, germination percentage was maximum (71.67%) for seed treated with KNO3 150 ppm which was 16.67% higher than untreated seeds (Figure 2a). The seed germination % under greenhouse condition was found maximum (73.33%) for seed source SD, treated with TU150 ppm which was about 18% higher than control (55%), for BN seeds the germination % was highest in KN150 ppm which was 25% higher than control (28.33 %) (Figure 2b).

Mean Germination Time (MGT)

The MGT was also found significantly affected by the seed source and the interaction between seed source and treatments also found significant (Table

Table 1. Two-way ANOVA showing the effect of seed source and plant growth regulators pre-treatment on the germination parameters of *S. obvallata* grown under seed germinator and greenhouse condition.

	Germination%		MGT		SOG	
	F	P	F	P	F	P
Treatments	16.22	<0.000	10.73	<0.000	16.29	<0.000
Source	64.67	<0.000	54.48	<0.000	1.54	<0.000
Treatments × Source	9.67	<0.000	18.91	<0.000	24.20	<0.000

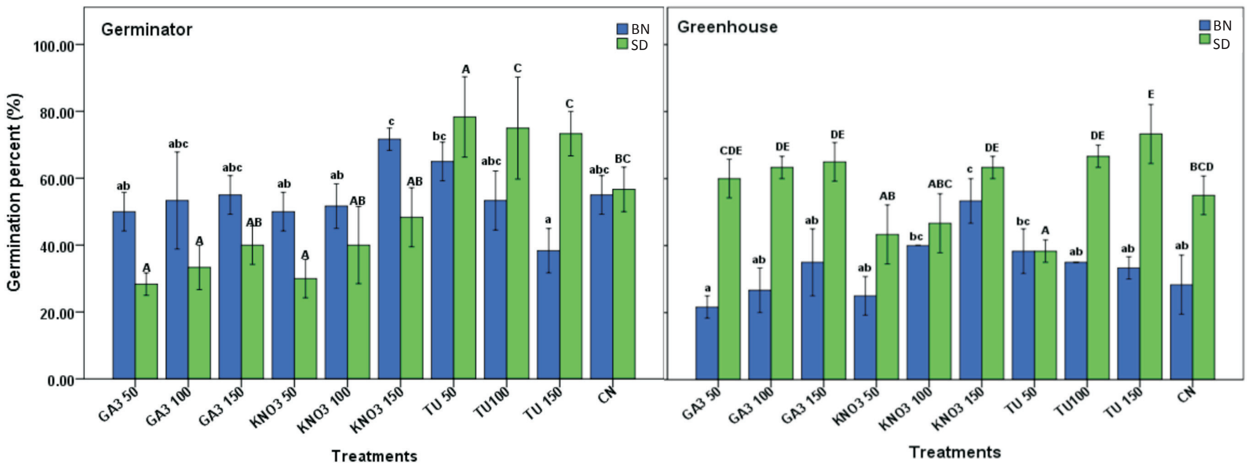


Fig. 2. Effect seed source (SD – Sundardunga and BN - Bedni) and different pre-treatments on seed germination % in germinator (a) and greenhouse (b). Similar alphabets (Capital letters for SD, small letters for BN) showed non-significant difference among the treatments.

1). Seeds treated with pretreatments showed lower MGT value under SG, the seeds from SD showed lowest MGT value (2.27) in treatment KN 100 ppm, which was 6 days earlier than control, for BN seeds the MGT value was lowest for TU 150 ppm which was approx 3 days earlier than control (Figure 3a). Under GH condition Seeds from SD showed lowest value (4.78) of MGT in seeds treated with KN 100 ppm followed by TU 50 ppm (6.40), while in control it was 9.15 days (Figure 3b). Seeds from BN did not showed any major difference in MGT under greenhouse condition.

Speed of Germination (SOG)

The SG was found significantly affected by treatments and seed source. The combined effect of these factors showed significant effect on SG (Figure). The highest SOG under SG was found in KN 150 ppm and TU 50 ppm of BN and SD treated seeds respectively, while in GH condition the SOG was maximum in KN 150 ppm and TU 150 ppm respectively in BN and SD treated seeds (Figure 4b).

Survivability of Seedlings

Seed grown under both SG and GH showed significant variation in survivability in seeds from SD and BN. After complete germination the seedlings were transplanted in field nursery for further study. After 60 days of transplantation it was observed that for BN seeds maximum survivability was found in KN

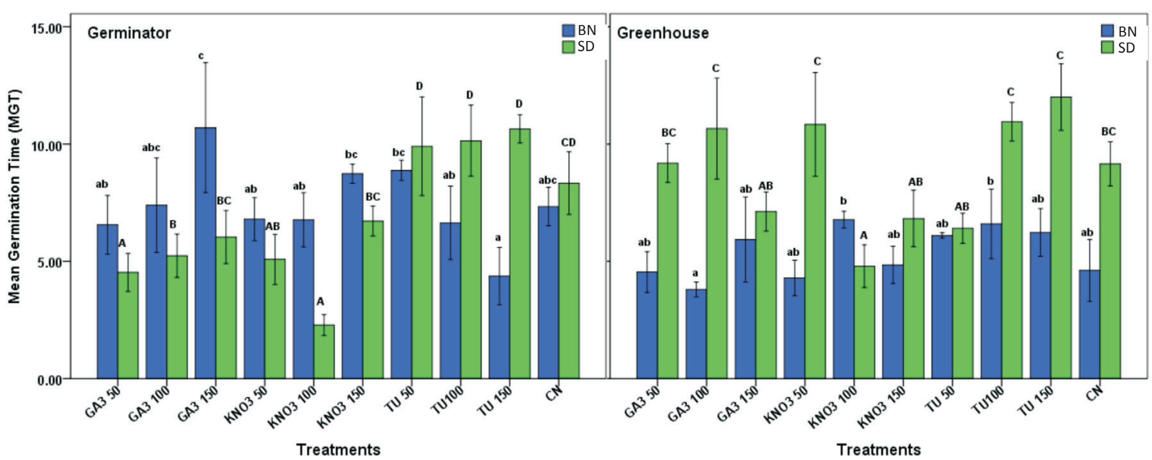


Fig. 3. Effect of seed source (SD and BN) and different Pre-treatments on Mean germination time (MGT) in germinator (a) and greenhouse (b). Similar alphabets (Capital letters for SD, small letters for BN) showed non-significant difference among the treatments.

150 ppm (32%) while for SD seeds it was maximum for TU 50 ppm (45%) under SG condition. Under GH condition maximum survivability was found in KN 150 ppm (45%) treated seeds of BN and TU 150 ppm (64%) for SD seeds. Our results showed that the survivability is significantly higher in pretreated seeds for both BN and SD seeds. Seeds without treated were unable to improve survivability for both seed sources (Table 2).

Seedling length and fresh biomass of seedlings

The morphological characters i.e. length and fresh biomass, of seedling were recorded and found that the seedling length of SD seeds was 38.8% (t=6.98, $p<0.001$) and 49% (t=7.49, $p<0.001$) higher than BN seeds under germinator and greenhouse condition respectively. The seedling biomass of SD seeds were also found 22.8% (t=6.63, $p<0.01$) and 29.9% (t=6.56, $p<0.01$) higher under SG and GH condition respectively. The seedling length was positively correlated with seed mass under SG (r = 0.65, $p<0.01$) and GH (r=0.74, $p<0.001$) condition (Figure 5). The seedling biomass was also positively correlated with seed mass under SG (r = 0.56, $p<0.01$) and GH (r=0.69, $p<0.001$) condition (Figure 6).

Discussion

Alpine ecosystem is an important biome which harbors 10000 plant species, temperature is a major limiting factor there, so the endemic plant of alpine regions are required to face harsh climatic condition and complete their life cycle in very short growing season (Jaganathan *et al.*, 2019). Seed mass and seed size play an important role in such harsh climatic condition in juvenile phase establishment

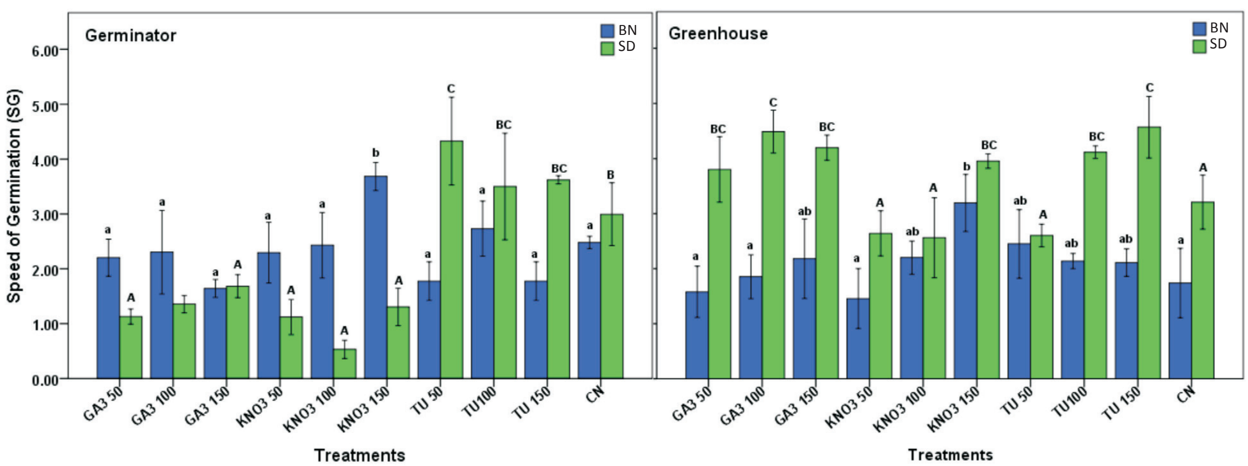


Fig. 4. Effect of Seed source (SD and BN) and different Pre-treatments on Speed of Germination (SOG) in germinator (a) and greenhouse (b). Similar alphabets (Capital letters for SD, small letters for BN) showed non-significant difference among the treatments

Table 2. Effect of different pre-treatments on survivability of *S. obvallata* seedlings after 60 days of transplantation of both seed germinator and greenhouse conditions.

Treatments	VN		SD	
	Under SG	Under GH	Under SG	Under GH
GA_3 50ppm	28	19	21	47
$GA_3$100ppm	25	25	27	50
$GA_3$150ppm	20	30	30	50
KNO_3 50ppm	29	20	25	30
KNO_3 100ppm	22	35	22	34
KNO_3 150ppm	32	45	25	47
TU 50ppm	28	30	45	30
TU 100ppm	30	27	32	50
TU 150ppm	20	22	40	64
CN	25	22	30	40

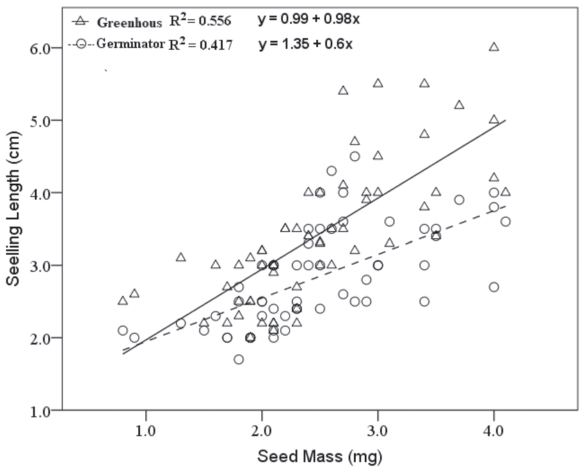


Fig. 5. Relationship between seedling length and seed mass for plants grown in two different conditions. Symbol round circle depicting seedling in germinator condition and triangle for greenhouse condition.

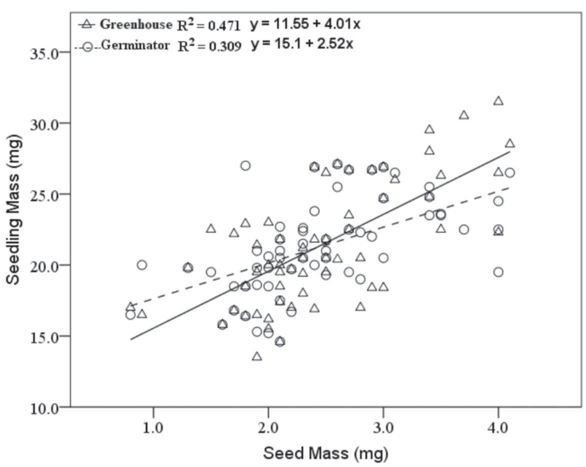


Fig. 6. Relationship between seed mass and seedling biomass in two different condition, open round circle for germinator and triangle for greenhouse condition.

(Upadhaya *et al.*, 2007). The storage food in seed governs germination, survival and growth of seed (Tripathi *et al.*, 1990). Investments of perennial plants into its offspring determine the seed size. Seed size is also an important factor for seedling growth and its future size (Olejniczak *et al.*, 2018). The present study showed significant variation in seed size and mass of two alpine regimes. The seeds from higher elevation are bigger and heavier, it may be due to plants from higher alpine region are well adapted to cold environmental conditions and low temperature favor its growth and development. The formation of the heavier seeds may be an adaptation for seedling establishment at harsh climatic sites. The heavier seeds may enhance germination due to larger embryo and more reserve energy which further utilized in the efficient mobilization of reserve materials (Parker *et al.*, 2006). The seed mass can also be determined by soil nutrient content, higher nutrient concentration often leads to the large and heavier seeds, the seed size and weight are the indicators of seed quality. The seed source determine the seed mass which influence the seed germination percentage which coincides with the work of (Loha *et al.*, 2006), who opined that seed germination capacity is due to provenance effect. Similarly, (Gosh *et al.*, 2011) also revealed the influence of seed source on germination performance. Seed mass is an important attribute of plants; it can govern seedling growth and its ability to compete (Rose *et al.*, 2003). The germination percentage was found enhanced by the application of PGRs for both seed source, the effect of PGRs was significantly depended on seed source (Table 1). There are number of studies (Elhindi *et al.*, 2016; Kandari *et al.*, 2012; Butola *et al.*,2004) reported germination enhancement in PGRs treated seeds. Seeds from SD showed enhanced germination in TU treated seeds, the TU application enhance the plant growth and development by increase the nutrient uptake and act as a enzyme regulator, TU is a good donor of sulphur atoms in biological reaction (Randle *et al.*, 1993; Shanu *et al.*, 2013). The seed mass was positively correlated with seedling length and biomass, heavier seed play important role in germination, growth and seedling establishment (Baskin *et al.*, 1998; Mole *et al.*, 2004; Lehtila *et al.*, 2005). The seed mass is an important factor that determines growth during juvenile phase at resource limited conditions (Khan 2004; Upadhaya *et al.*, 2007). The higher seed reserves play beneficial role in the development of more photosynthetic tissue and which further induce biomass (Foster, 1986). The seeds from SD showed greater survivability than BN, lighter seeds may produce more seedlings faster than heavier seeds but the chances of survivability at seedling and later stages would be greater for heavier seeds (Westoby *et al.*, 1992; Baraloto *et al.*, 2005; Baskin *et al.* 1998) because of greater storage food which help in the growth and development of plant. The treatment of TU showed most stable and high survivability that may be due to its effect on total uptake of N, P and K and which enhance mobilization of essential nutrient through lighter the microbial population in soil (Balai *et al.,* 2012).

S. obvallata is a critically endangered medicinal plant species limited to higher Himalayan region. The plant has immense value in traditional system of medicine and considered as sacred plant. Due to intense exploitation of its flower before seed setting, the species is facing threat of habitat degradation day by day. There are limited attempts recorded on development of conservation strategy of this species through seeds or other propagation means so far. Therefore, the results of the present study will be useful in survival of the species in long run.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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