Effect of Different Temperatures on the Seed Germination of Willow Leaf Seabuckthorn – *Hippophae salicifolia*

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Introduction

Hippophae salicifolia D. Don (Vernacular - Chuk. Tarwa) is a deciduous tree species restricted to the Himalayan region, between 1500-3500 m a. m. s. l. (Hooker, 1894 and Gaur 1999). It has been reported as one of the best species of genus Hippophae in terms of high quality fruit, high yield and less thorns (Lu *et al.*, 2001). There are total five species based on morphological variations viz. *H. rhamnoides* L., *H. salicifolia* D. Don, *H. neurocarpa* Liu & He, *H. tibetana* Schlecht and *H. goniocarpa*. The main species of Seabuckthorn distributed in India are *H. rhamnoides*, *H. salicifolia* and *H. tibetana*. Out of these species, only two species, *i.e.*, *H. salicifolia* D. Don and *H. tibetana* S. are reported in Uttarakhand (Yadav *et al.*, 2006 a). Among them *H. salicifolia* is the most common and widely distributed species and also called as willow leaf or arboreal is reported to exist in abundance in three districts of Uttarakhand viz., Uttarkashi, Chamoli and Pithoragarh (Yadav *et al.*,2006 b).

The uses of Seabuckthorn are manifold and include its use as fence, fuel, fodder, fruit and medicines. It is used for protection against wild animal and is used as bio-fence around houses and cultivated fields. This species has great ecological significance as its roots possess excellent soil binding properties. Frankia present in its root nodules fixes atmospheric nitrogen @180kg/ha/annum. Natural forest of Seabuckthorn can yield 750-1,500 kg of berries/ha and berries are the rich source of vitamin C, caretonoids, minerals, vitamin B, vitamin E and vitamin K. Seeds contain high quality oil which has many bioactive substances (Lu,1992).

The fruits have a distinctive sours taste and a unique aroma reminiscent of pineapple. The local peoples of Uttarakhand state who leaved nearby, the habitat of *H. salicifolia*, they used berries for pickles. The berries remain on the small tree branches all winter until eaten by birds. Scientifically the quality of fruit was recorded as a rich source of vitamins, and used in preparations of various products including local beverages (Gaur,1999).

In the present study, the main objective was to determine most appropriate temperature requirements for optimum germination of *H.salicifolia*. As appropriate temperature is probably the most important factor in regulating germination (Nerson, 2007). Temperature affects the germination capacity, the germination rate and the germination frequency alongside the incubation time (Kocabas *et al.*, 1999). Germination speed usually increases until the temperature reaches 30-35^{o}C (Roberts and Ellis, 1989). Temperature has significant effects on the onset, potential and rate of germination (Flores and Briones, 2001). The thermal limits for germination are defined by the minimum (Tm), optimum (To) and maximum (TM) temperatures which can

determine some of the ecological limitations for the geographic distribution of the species. Optimum temperature is the temperature value which results in the highest germination speed (Hakansson *et al.*, 2002). Optimum temperatures produce most rapid seed germination and plant growth both.

Temperature regulates germination in three ways (Bewley & Black, 1994): 1) by determining the capacity and percentage of germination; 2) by eliminating primary and/or secondary dormancy; and 3) by inducing secondary dormancy. The optimal germination temperature for most seeds which are not in dormancy is 25 to 30°C. However, temperature ranges for germination depend largely on the (1) period of the year in which plants complete their life cycle and (2) geographic origin of the species (Besnier, 1989). Thus, seeds usually require a thermal periodicity to germinate: a cold season (of activation), between 0 and 10 °C (stratification), alternating with another one where the embryonic axis grows at intermediate temperatures (20-30 °C). In fact, low temperatures can break dormancy of some seeds (*e.g.* woody plants, cereals), and are necessary for germination at higher temperatures (Bewley & Black, 1982). High temperatures sometimes reduce dormancy of some seeds but induce secondary dormancy in others. Therefore, the response of seeds to these conditions can be essential for the regeneration of communities after a perturbation.

Materials and methods

The main objective of present study was to determine most effective temperature requirements for germination of the study species under laboratory conditions which would contribute to know the required temperature conditions necessary for optimum germination of *H.salicifolia*. After seed germination in the laboratory, seedlings were transplanted to the natural environment.

The investigation was carried out in three Provenances of Uttarakhand State in India viz. Uttarkashi (P1), Chamoli (P2) and Pithoragarh (P3). The geographic range of the provenances selected for seed collection varied from 30° 03 to 31° 34' N latitude, 74° 30' to 80° 13' E longitude and 1949 to 3212 m altitude. Seed samples were collected from randomly selected fruiting branches from as many plants as possible in healthy looking populations.

All seeds were surface sterilized by immersion in 1% sodium hypochlorite for 5 min, and washed afterwards with distilled water. Thereafter, petri dishes 10 cm in diameter were prepared with filter paper (both previously sterilized). 10 ml of distilled water was added. Experiments were conducted in order to investigate germination behavior of *H. salicifolia* seeds subjected to different temperatures *i.e.* 15 °C, 20 °C, 25 °C, 30 °C and ambient temperature varying from 15°- 35 ° C and dishes were sampled daily during 15 days. Seeds were considered germinated when the radicle protruded at least 2 mm from the seed coat (Jeffrey *et al.*, 1987). Germinated seeds and rotted seeds were counted and discarded at 24 h intervals until no germination occurred on 4 consecutive days (Samimy *et al.*, 1987). Distilled water was added whenever moisture loss was detected.

There were 5 treatments in this experiment including the control (ambient temperature). The experiment was undertaken in completely randomized design with four replication in each treatment and twenty five seeds per replication. Results were expressed as germination percentage which was the percentage of live seeds that had germinated at the end of test.

Response Index (RI) was calculated as per the formula given by (Richardson and Williamson 1998) for the magnitude of inhibition versus stimulation by different temperatures on seed germination and radicle/plumule growth of *H. salicifolia*.

$$RI = (T/C)-1 \times 100$$

Where,

T = treatment, C = control

Fruiting in salicifolia

Germinated seeds

Radicle and Plumule length

Effects of different temperatures on *H.salicifolia* seeds

Results

The study was conducted in the Forest Research Institute (30° 20'N, 78° 01' E; 670 m amsl) in Dehradun, Uttarakhand, India during the year 2009. The metrological data is given in Table 1.

The present study revealed that the maximum germination percentage was observed in P2 under 25°C (91.04%) and minimum germination observed in 30°C (16.67 %) in P3. The results reveals that 15 °C, 20°C and 25 °C temperature have positive influence on the seed germination but 30 °C temperature showed negative influence on seed germination in all the three provenances under study(Table 2).

The effects of temperature on radicle growth showed that maximum radicle length was observed in 25 °C (3.68cm) in P2 and minimum under 30 °C (0.56 cm) in P3. P3 also showed negative influence of radicle growth in 15, 25 and 30 °C temperatures. 30 °C temperature showed negative influence on radicle growth in all the three provenances under study (Table 3).

The effects of temperature on Plumule growth showed that maximum plumule length was observed in control (2.62 cm) and minimum under 30 °C (0.66 cm) in P3. P1 and P3 showed negative influence as comparison to control on all the temperatures under study(Table 4).

Table 1 : Weather Data of Forest Research Institute , Dehradun of the Year, 2010

Month	Temperature (°C)			Vapour (mm of Hg) Pressure		Relative Humidity (%)		Rainfall (mm)/ month	Evaporation open pan (mm)/ month	Bright sunlight (hrs)/day	Mean wind velocity (Km/hr)
	Max	Min	Mean	At 0719 hrs	At 1419 hrs	At 0719 hrs	At 1419 hrs				
February	23.1	06.7	14.5	07.7	08.1	97	40	60.5	1.9	7.6	2.5
March	30.6	12.3	21.2	10.9	10.2	98	32	01.7	3.8	8.8	2.8
April	36.6	15.3	25.8	10.9	09.0	61	20	0.5	6.8	8.2	3.5
May	36.4	19.5	27.3	14.4	12.2	59	30	025.0	7.2	8.4	3.8
June	36.1	20.7	27.5	17.2	15.2	68	39	090.6	6.3	7.3	3.7
July	30.7	22.9	26.3	21.8	22.4	93	73	661.4	2.7	3.6	2.8
August	29.5	23.0	25.6	21.9	22.8	95	79	952.4	1.8	3.3	2.5
September	28.5	20.7	24.0	19.4	20.7	95	77	856.7	2.1	5.0	2.6
October	29.7	14.5	22.1	13.6	15.4	95	53	008.3	2.5	7.5	2.2

Table 2 : Influence of different temperatures on seed germination of *Hippophae salicifolia*

Treatment	P1 (%)	RI	P2 (%)	RI	P3 (%)	RI
Ambient	59.17	-	84.33	-	66.66	-
15°C	76.25	28.86	84.50	0.20	76.50	14.76
20°C	79.33	34.07	86.58	2.57	82.42	23.64
25°C	80.83	36.61	91.04	7.96	87.92	31.89
30°C	23.33	-60.57	34.17	-59.48	16.67	-74.99

RI = Response Index

Table 3. Radicle growth (cm) and Response index (%) of *Hippophae salicifolia* under different temperatures

Treatment	P1	RI	P2	RI	P3	RI
Ambient	1.79	-	2.14	-	1.81	-
15°C	2.12	18.43	2.47	15.42	1.29	-28.72
20°C	1.90	6.14	2.82	31.77	2.10	16.10
25°C	3.10	74.30	3.68	71.96	1.61	-11.04
30°C	0.89	-50.27	1.48	-30.84	0.56	-69.06

Table 4. Plumule growth (cm) and Response index (%) of *Hippophae salicifolia* under different temperatures

Treatment	P1	RI	P2	RI	P3	RI
Ambient	2.10	-	2.36	-	2.62	-
15°C	1.64	-21.90	2.27	-3.81	1.73	-33.96
20°C	1.67	-20.47	2.12	-10.16	2.18	-16.79
25°C	1.98	-5.71	2.62	11.01	2.53	-3.43
30°C	1.02	-51.42	1.98	-16.10	0.66	-74.80

Discussion

H. salicifolia seeds germination started 7-10 days after sowing in 25°C to 20°C temperature respectively but seeds in 15°C started to germinate after 15 days. As highest and quickest germination occurred at 25°C. Therefore 25°C can be considered optimum temperature for seed germination of *H. salicifolia* under laboratory conditions. The present results were in consonance with the findings of Rongsen (1992) in *H.salicifolia*. The lower and upper temperature thresholds for germination of *H.salicifolia* were not encountered in this study, but very less germination prevailed at temperatures lower than 15 °C, and germination declined at temperatures higher than 30 °C. However, effect of low and high temperatures among seeds in a population or in germination sensitivity were observed by others (Orozco-Segovia *et al.* 1996; Kebreab & Murdoch 2000; Grundy *et al.* 2000), and changes in both the upper and lower temperature limits for germination are often associated with the imposition and release of dormancy (Kruk and Benech 2000).

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