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Effect Of Different Temperature Regimes And Chemicals On Seed Germination Behaviour Of *Hippophae Salicifolia* D. Don

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Abstract: *Hippophae* spp. (commonly known as Seabuckthorn) is a hardy deciduous shrub of family Elaeagnaceae, native to the temperate regions of Europe and Asia. *Hippophae* is considered to include seven species and eight subspecies, one of which is *Hippophae salicifolia* D. Don is also found in Indian Himalayan region. It is important for medicinal use in traditional to modern system of medicines *e.g.*- cough, bronchitis and expectorant. The present investigation was carried out to improve seed germination. We applied different chemicals including GA3 (5mM, 10mM, 15mM, 20mM), KNO3 and thiourea (50 mM, 75 mM, 100 mM, 125 mM) at room temperature for 24h. Treated seeds placed in Petri plates containing moistened Whatman filter paper. Petri plates were kept at different temperature regimes (room, 20°C, 25°C and 30°C) in incubator and moistened as per need with distilled water. A set of seeds without pre-sowing treatments were considered as control. The experiment was carried out in Factorial Control Randomized Design with three replications. Various germination parameters were observed and results indicated that among different treatments combinations; T4U4, (temperature 30°C × thiourea @ 100 mM) was found effective in all seed germination parameters but negative effect of this temperature were seen on survival of seedlings therefore treatment combination T3U4, (temperature 25°C × thiourea @ 100 mM) was found more suitable for early germination (8 days), mean daily germination (0.85 percent seeds/day) and germination percent (94.67).

Key words: Seed germination • Chemicals • Survival • GA3 • KNO3 • Thiourea

Introduction

Hippophae is a Latin word derived from the two words "hippo" meaning-horse and "phaos" meaning- sparkle. In ancient times, the leaves and twigs of *Hippophae* were known as a feed to racehorses for weight gain and sparkling coat; therefore, its name is *Hippophae* (Suryakumar and Gupta 2011). In India, *Hippophae* spp. has been found in cold desert and other regions of Himalayas, comprising the states of Himanchal Pradesh, Ladakh, Uttarakhand, Sikkim and Arunachal Pradesh. *H. salicifolia* is the well- known among them and broadly distributed in Hill regions of Uttarakhand.

It is one of the important medicinal plants with nutritious fruits which are having huge medicinal and pharmaceutical importance used for the treatments of cough, bronchitis, expectorant, etc. The fruits of *H. salicifolia* are very rich in nutrients and other vital bio-synthetics or biologically active compounds. The bark of plant is brown or black in colour with a rough appearance. The leaves are alternate, narrow, and lanceolate with a greyish appearance. Dormancy is a condition where seed will not germinate even when the environmental conditions (water, temperature and aeration) are permissive for germination. Seed dormancy is mainly due to inability of embryonic tissues to emerge out through the seed coat. This may be due to either hardness of the seed coat, presence of germination inhibitors (seed Coat-enhanced or physiological dormancy) or dead embryo (embryo-enhanced dormancy). Seed germination is one of the important method for multiplication in this species, but sometimes seeds show dormancy that limits propagation. Seed germination can be improved by the pretreatments with various chemicals and growth regulators. Nitrogenous compounds, like nitrate, thiourea and growth regulators have been successfully implicated in breaking both the innate and environmental imposed seed dormancy and promotion of seed germination. GA3, KNO3 and thiourea, etc. widely used for breaking seed dormancy and promoting seed germination.

Materials and Methods

The mature healthy seeds of *Hippophae salicifolia* D. Don. were collected during October 2020 from farm of Department of Medicinal and Aromatic Plants, College of Horticulture, Veer Chandra Singh Garhwali, Uttarakhand University of Horticulture and Forestry, Bharsar, Pauri Garhwal, Uttarakhand. Immediately after collection, seeds were cleaned manually, dried for one week in sunlight and stored at room temperature until use. Seeds were disinfected by immersing in 1% $HgCl_2$ solution for 1 min followed by rinsing thoroughly with distilled water. The sterilized seeds were soaked for 24hr in different chemicals solutions of desired concentration of GA3 (5mM, 10mM, 15mM, 20mM), KNO3 and thiourea (50 mM, 75 mM, 100 mM, 125 mM) at room temperature. Treated seeds placed in Petri plates containing moistened Whatman filter paper. Petri plates were kept at different temperature regimes [(room temperature), 20°C, 25°C, 30°C)] in incubator and moistened as needed with distilled water. A set of seeds without pre- sowing treatments were considered as control. The experiment was carried out in Factorial Control Randomized Design with three replications. Seed germination was recognized by emergence of radical, while whole experiment was monitored up to 30 days. Data on various parameters of seed germination was recorded as below:

Days taken for initial germination

The period from the date of sowing to the emergence of first radicle was considered as the time taken for the initiation of seed germination.

Mean daily germination (MDG):

$$\text{Mean daily germination} = \frac{\text{Total Number of germinated seed}}{\text{Total nunber of days}}$$

Germination per cent:

$$\text{Germination \%} = \frac{\text{Number of seed germinated}}{\text{Number of seeds kept for germination}}$$

Statistical analysis

The statistical analysis was carried out for each observed character under the study using MS-Excel, OPSTAT. The data obtained during the investigation were analyzed by using standard statistical procedure in the Factorial Complete Randomized Design (CRD).

Results and Discussion

Among different temperature regimes with response to GA3 concentrations; the minimum (9.60) days taken for initial germination at temperature T4 (30°C). This result might be due to temperature play an important role in seed germination by affecting the metabolism involved in onset of germination (Verma *et al.*, 2010). Temperature can affect germination by affecting dormancy or the germination process with respect to different concentrations of GA3; minimum (9.75) days taken for initial germination in treatment G5 (GA3 @ 20 mM).It might be attributed to fact that GA3 helps in breaking the seed dormancy which results in early and enhanced seed germination due to the diffusion of endogenous auxin and gibberellins like substances (Gurung *et al*., 2014; Singh *et al*., 2016).The result also revealed the interaction effect of temperature and GA3 indicated that minimum (8.67) days taken for initial germination in the treatment combination T4G5 (temperature 30°C ×GA3 @ 20mM).

Among different temperature regimes with response to KNO3 concentrations; the minimum (9.07) days taken for initial germination at temperature T4 (30°C) whereas, the maximum (12.40) days under temperature T1 (room temperature). Temperature is an important factor during the seed germination process (Ooi *et al*., 2012). With respect to different concentrations of KNO3; minimum (8.75) days taken for initial germination in treatment K5 (KNO3 @ 125 mM) while, the maximum (11.92) days in K1 (control). It might be due to that KNO3 has been used as a growth regenerating and germination- stimulating substance. It enhance seed germination by creating a balance between hormonal ratios in the seed and reducing the growth preventable substances like abscisic acid. The result also revealed the interaction effect of temperature and KNO3 indicated that minimum (7.67) days taken for initial germination in the treatment combination T4K5 (temperature 30°C× KNO3 @ 125 mM) whereas, the maximum (14.67) days in the treatment combination T1K1 (control).

Among different temperature regimes with response to thiourea concentrations; minimum (8.27) days taken for initial germination at temperature T4 (30°C). With respect to different concentrations of thiourea; minimum (8.17) days taken for initial germination in treatment U4 (thiourea @ 100mM). Improved seed germination under thiourea treatment is an agreement with reports of many other species. The result also revealed the interaction effect of temperature and thiourea indicated that minimum (7.33) days taken for initial germination in the treatment combination T4U4 (temperature 30°C × thiourea @ 100 mM).

Among different temperature regimes with response to GA3 concentrations; maximum mean daily germination (0.78) percent were recorded at temperature T4 (30°C). Temperature regulates germination in three ways 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 of the seeds; which are not in dormancy is 25°C to 30°C.With respect to different concentrations of GA3; the maximum mean daily germination (0.75) percent were recorded in treatment G5 (GA3 @ 20 mM). It might be due to that application of GA3 is known to promote germination by breaking dormancy in a wide range of seeds. The result of interaction effect of temperature and GA3 indicated that maximum mean daily germination (0.88) percent in the treatment combination T4G5 (temperature 30°C × GA3 @ 20mM). These findings are in conformity with those of Butola e*t al*. (2007) who found that GA3 (150 mM) was effective in stimulating germination (70%) and reducing MGT (17days) over control in *Hypericum perforatum.*

Among different temperature regimes with response to KNO3 concentrations; maximum mean daily germination (0.83) percent were recorded at temperature T4 (30°C) while, the minimum mean daily germination (0.55) per cent under T1 (room temperature).

With respect to different concentrations of KNO3; maximum mean daily germination (0.79) percent were recorded in treatment K5 (KNO3 @125 mM) whereas, minimum mean daily germination (0.63) percent in K1 (control). Increase in KNO3 concentrations resulted in corresponding increase in germination and seedling growth, as it plays a critical role in increasing physiological efficiency of the seeds (Bhargava and Banerjee, 1994). The result of interaction effect of temperature and KNO3 indicated that maximum mean daily germination (0.97) percent in the treatment combination T4K5 (temperature 30°C × KNO3 @ 125 mM) while, minimum mean daily germination (0.46) percent in the treatment combination T1K1 (control).

Among different temperature regimes with response to GA3 concentrations; maximum germination (85.27) percent were recorded at temperature T4 (30°C). Temperature is one the most important environmental factor determining the success of germination and all seeds need proper temperature for germination. Generally, low temperature significantly delayed the germination (Pourreza and Bahrani, 2012). With respect to different concentrations GA3; the maximum germination (85.67) percent were recorded in treatment G5 (GA3 @20 mM). The increased germination percentage in GA3 treated seeds might be that GA3 enhances the germination of seeds exhibiting physiological, morphological or morph physiological dormancy (Ganai and Nawchoo, 2002; Shivakumar *et al*., 2006). The result of interaction effect of temperature and GA3 indicated that maximum germination (89.33) percent in the treatment combination T4G5 (temperature 30°C × GA3 @ 20mM). Similar results were obtained by Bhatt *et al*. (2005) who reported that GA3 as the best treatment for improving seed germination in *Swertia angustifolia*. Similar results were also found by Rawat *et al*. (2008) in *Cupressus torulosa*.

Among different temperature regimes with response to KNO3 concentrations; maximum germination (86.87)percent were recorded at temperature T4 (30°C) whereas, the minimum germination (78.13) per cent under T1 (room temperature). With respect to different concentrations of KNO3; the maximum germination (88.33) percent were recorded in K5 (KNO3 @125 mM) while, minimum germination (77.17) per cent in K1 (control). Potassium nitrate was very effective in breaking seed dormancy of many species (Previero e*t al*., 1996). Seed treatments with KNO3 is also known to enhance the germination percentage (Silvertown and Lovett Doust, 1993). The result of interaction effect of temperature and KNO3 indicated that maximum germination (92.00) percent in the treatment combination T4K5 (temperature 30°C × KNO3 @ 125 mM) whereas, minimum germination (66.67) percent in the treatment combination T1K1 (control).Results obtained on this aspect are in agreement with El-Keblawy and Gairola (2016) in desert annuals

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Table 1. Effect of different temperature regimes and chemicals on days taken for initial germination

Temperature (T)	**Gibberellic acid concentration (G)**						**Potassium nitrate concentration (K)**						**Thiourea concentration (U)**					
	G_1	G_2	G_3	G_4	G_5	Mean (T)	K_1	K_2	K_3	K_4	K_5	Mean (T)	U_1	U_2	U_3	U_4	U_5	Mean (T)
T_1	14.67	14.33	13.33	10.33	10.00	12.53	14.67	14.33	13.33	10.00	9.67	12.40	14.67	14.00	13.33	9.00	11.33	12.47
T_2	12.67	12.00	11.67	11.33	11.00	11.73	12.33	10.67	10.33	9.67	9.00	10.40	11.00	10.00	9.00	8.33	8.67	9.40
T_3	11.33	10.67	10.33	9.67	9.33	10.27	10.67	10.33	9.67	9.33	8.67	9.73	10.00	9.00	8.67	8.00	8.33	8.80
T_4	10.33	10.00	9.67	9.33	8.67	9.60	10.00	9.67	9.33	8.67	7.67	9.07	9.33	8.67	8.33	7.33	7.67	8.27
Mean	12.25	11.75	11.25	10.17	9.75		11.92	11.25	10.67	9.42	8.75		11.25	10.42	9.83	8.17	9.00	
Factors	SE(d)			CD			Factors		SE(d)		CD			Factors			SE(d)	CD
Temperature (T)	0.41			0.83			Temperature(T)		0.34		0.69			Temperature(T)			0.37	0.76
GA_3 Conc.(G)	0.46			0.93			KNO_3Conc. (K)		0.38		0.78			Thiourea conc. (U)			0.42	0.85
Interaction (T×G)	0.91			1.86			Interaction (T×K)		0.77		1.56			Interaction (T×U)			0.84	1.69

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Table 2. Effect of different temperature regimes and chemicals on mean daily germination (MDG % number of seeds germinated per day)

Temperature (T)	**Gibberellic acid concentration (G)**						**Potassium nitrate concentration (K)**						**Thiourea concentration (U)**					
	G1	G2	G3	G4	G5	Mean(T)	K1	K2	K3	K4	K5	Mean(T)	U1	U2	U3	U4	U5	Mean(T)
T1	0.46	0.52	0.54	0.60	0.62	0.55	0.46	0.52	0.54	0.62	0.63	0.55	0.46	0.51	0.54	0.65	0.61	0.56
T2	0.61	0.69	0.71	0.72	0.73	0.69	0.60	0.70	0.72	0.73	0.73	0.69	0.60	0.70	0.74	0.76	0.72	0.70
T3	0.72	0.73	0.74	0.75	0.76	0.74	0.73	0.74	0.76	0.79	0.83	0.77	0.74	0.80	0.81	0.85	0.82	0.80
T4	0.73	0.74	0.75	0.802	0.88	0.78	0.74	0.77	0.81	0.89	0.97	0.83	0.76	0.82	0.89	1.26	0.86	0.92
Mean	0.63	0.67	0.69	0.72	0.75		0.63	0.68	0.70	0.76	0.79		0.64	0.71	0.74	0.88	0.75	

Factors	SE(d)	CD	Factors	SE(d)	CD	Factors	SE(d)	CD
Temperature(T)	0.02	0.05	Temperature(T)	0.02	0.04	Temperature(T)	0.02	0.05
GA_3 Conc.(G)	0.03	0.06	KNO_3 Conc. (K)	0.02	0.04	Thiourea conc. (U)	0.03	0.06
Interaction (T×G)	0.06	0.12	Interaction (T×K)	0.04	0.08	Interaction (T×U)	0.06	0.11

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Table 3. Effect of different temperature regimes and chemicals on germination per cent

Temperature (T)	Gibberellic acid concentration (G)						Potassium nitrate concentration (K)						Thiourea concentration (U)					
	G_1	G_2	G_3	G_4	G_5	Mean (T)	K_1	K_2	K_3	K_4	K_5	Mean (T)	U_1	U_2	U_3	U_4	U_5	Mean (T)
T_1	66.67	76.00	78.67	81.33	82.67	77.07	66.67	77.33	80.00	82.67	84.00	78.13	66.67	78.67	81.33	86.67	78.67	78.40
T_2	77.33	78.67	81.33	82.67	84.00	80.80	78.67	80.00	82.67	84.00	86.67	82.40	77.33	81.33	85.33	89.33	85.33	83.73
T_3	80.00	82.67	84.00	85.33	86.67	83.73	81.00	84.00	85.33	86.67	90.67	85.53	81.33	85.33	86.67	94.67	90.67	87.73
T_4	81.00	84.00	85.33	86.67	89.33	85.27	82.33	85.33	86.67	88.00	92.00	86.87	82.67	86.67	88.00	96.00	93.33	89.33
Mean	76.25	80.33	82.33	84.00	85.67		77.17	81.67	83.67	85.33	88.33		77.00	83.00	85.33	91.67	87.00	
Factors	SE(d)			CD			Factors		SE(d)		CD	Factors		SE(d)		CD		
Temperature (T)	2.04			4.13			Temperature (T)		1.40		2.86	Temperature (T)		1.18		2.39		
GA_3 conc. (G)	2.28			4.62			KNO_3 conc. (K)		1.58		3.19	Thiourea conc. (U)		1.32		2.68		
Interaction (T×G)	4.55			9.19			Interaction (T×K)		3.15		6.37	Interaction (T×U)		2.63		5.32		

Conclusion

On the basis of result obtained from present investigation, it may be concluded that among the different temperature regimes T4 (30°C) was most effective, with germination (96.00) percent but being a plant of temperate region negative effect of higher temperature were seen on the survival of seedlings. Therefore temperature T3 (25°C) were found suitable as it showed almost similar germination as in T4 (30°C) under study for all the observation that is (8.00) days taken for initial germination, mean daily germination percent (0.85), germination percent (94.67), germination energy percent (50.00), speed of germination percent seeds germinated per day (0.79), shoot length (3.45 cm) root length (1.16 cm) after 30 days and seedling vigour index-I (113.26). As regard to the different chemicals treatment U4 (thiourea 100 mM) was observed the best as compare to other chemical (GA3, KNO3) concentrations taken under study for influencing the germination behaviour, but increase in the thiourea concentration resulting in the decreases the germination behaviour. Therefore, the treatment combination T3U4 (temperature 25°C × thiourea @ 100 mM) most superior for seed germination in *Hippophae salicifolia*.

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