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Enhancement of Seed Germination by Pre-sowing Seed Treatments and Growing Media in *Berberis lycium* Royle

Sachin Verma*, Uday Kumar, Jayashree Behera and Vimal Chauhan Department of Silviculture and Agroforestry, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan, (Himachal Pradesh), India.

> (Corresponding author: Sachin Verma*) (Received 10 November 2021, Accepted 17 January, 2022) (Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: *Berberis lycium* (Kashmal) an evergreen shrub generally grows in Himalayan region, every part of this plant used in various medicinal preparations due to presence of berberine in rhizomes has marked an antibacterial effect and raw product used in various pharmaceutical industries. Due to its hard seed coat, seed remained dormant for long time results poor germination as now on the plant is on vulnerable stage just about to getting endangered. Therefore present study evaluates various pre-sowing seed treatment to reduce dormancy period and enhance germination viz. Immersion of seeds in hot water, H_2SO_4 and cow urine for different time interval which was later grown on Soil, Sand, Soil+Sand (1:1), Soil+Sand+FYM (1:1:1) out of which immersion of seeds in cow urine for six hours grown on Soil+Sand+FYM (1:1:1) medium resulted maximal seed germination (65.33%) while the minimal germination (33.33%) was observed on control (untreated seeds) grown on soil medium.

Keywords: Regeneration, germination, potting mixture, propagation.

INTRODUCTION

Berberis lycium Royle is a well-known medicinal plant commonly used in traditional herbal medicines and recipes for many ailments (Bhattacharjee, 2008). It is an evergreen shrub endemic to the Himalayas that belongs to the Berberidaceae family and the genus Berberis. It occurs in the Himalayan region between 850-3500 meters above mean sea level (AMSL) on moderate to steep slopes (Sharma et al., 2017). The flowers of the plant are bright yellow in colour which acquire bright red or purplish colour on ripening (i.e. fruits). The flowering season of Berberis lycium is from the month of April-May and September-October and fruiting season is from June-July (Gupta et al., 2015). According to IUCN Berberis lycium is having a vulnerable conservation status (Waseem et al., 2006). Every part of this plant, viz. roots, bark, stem, and fruits, have therapeutic value used in various medicinal preparations. B. lycium has been reported by various studies for its use in traditional system of medicine to treat infectious diseases along with fever, wounds (Aggarwal, 2006) diabetes, rheumatism, and gingivitis (Shabbir et al., 2012). Berberine present in rhizomes has marked antibacterial effects (Sood et al., 2012). It is utilized for fuel wood due to high calorific value of its wood. B. lycium, like many other wild fruit species, is

over-exploited in nature but not domesticated. For ecological restoration of denuded hill slopes, plantation of this species is considered an ideal choice (Verma *et al.*, 2020). The artificial regeneration in this species through seed gives little success as the seeds show around 34.00 per cent germination. There is enormous biotic pressure on this shrub species, which results in poor regeneration of this species (Kumari *et al.*, 2017). The main reason for the poor germination is seed dormancy, though their viability is 82.00 per cent (Bahuguna *et al.*, 1988).

Considering the importance of this vulnerable shrub species with poor seed propagation, a study was undertaken to study the effect of pre-sowing seed treatments and different growing media on germination behavior.

MATERIAL AND METHODS

The experiment related to the germination of the seeds of *Berberis lycium* Royle. were conducted in Majhgaon nursery of the Department of Silviculture and Agroforestry, Dr. YS Parmar University of Horticulture and Forestry during 2017-2018, located at 30° 5' N latitude and 76° 11' E longitude. The place lies at an altitude of 1100 to 1275 meters above mean sea level in the eastern direction of Solan town. The study area falls

Verma et al.,

Biological Forum – An International Journal 14(1): 1119-1122(2022)

in the moist sub-temperate zone, which experiences 1000 mm to 1300 mm precipitation annually, the major part of which is received during July and August (monsoon period). Winter showers, though common, are usually mild. Frost occurs recurrently from December to February. May and June are the hottest months, and December and January are the coldest.

The seeds were collected in the locality of the university campus at an altitude of 1100 to 1275 m during June, 2017. The collected seeds were subjected to drying under shade for 25 days, and all the foreign matter from the seeds was removed. Before sowing, the seeds were treated with fungicide to control fungal attacks. *Berberis lycium* Royle. Seeds were subjected to four different pre-sowing treatments comprises T_1 (Control), T_2 (Immersion in hot water (80 C) for 30 minutes), T_3 (Immersion in H_2SO_4 (80.00% conc.) for 5 minutes), T_4 (Immersion in cow urine for 6 hours). After pre-sowing treatment, the seeds were sown in root trainers placed under the poly house.

A potting mixture of soil, sand, and farm yard manure (FYM) in four different proportions was used to fill the root trainers comprised M_1 (Soil), M_2 (Sand), M_3 (Soil+Sand in ratio 1:1), M_4 (Soil+Sand+FYM in ratio 1:1:1). The pot filling mixture was thoroughly mixed in desired proportions before filling the pots. Containers were filled with the growing media after gentle tapping on the ground. After that, containers were kept on the ground. Watercan irrigation was done whenever required.

The data on germination parameters viz. germination percentage, germination capacity, germination energy, mean daily germination, and peak value were recorded and calculated using the following formula:

Germination percentage (%): According to Scott *et al.* (1984), the higher the germination percentage value, the greater the germination of a seed population.

 $\begin{array}{l} \text{Germination} \quad \text{percent} \quad (\%) = \\ \frac{\text{Total number of seeds germinated}}{100} \times 100 \end{array}$

Total number of seeds sown Germination capacity (%): Germination capacity (GC) of a seed lot was calculated as the cumulative number of seeds that had germinated at the end of the test period plus the number of un-germinated viable seeds at the end of the test expressed in percentage (Paul, 1974). Germination energy (%): Germination energy (GE) was calculated based on the percentage of the total number of seeds that had germinated when the germination reached its peak, generally taken as the highest number of germination in 24 hours period (Czabator, 1962).

Germination energy (%) Number of seeds germinated up

to time of peak germinated up

 $\frac{100}{\text{Total number of seeds sown}} \times 100$

Mean daily germination: According to Paul (1974), the cumulative percentage of seed germination at the end of the test is divided by the number of days taken from sowing to the end of the test or total percentage germination divided by total days in the test gives the mean daily germination.

Peak value: The maximum mean daily germination reached at any time during the period of the test gives the peak value (Paul, 1974).

The data of various germination characters studied in the present investigation were statistically analysed by the procedure of Completely Randomized Design (CRD) Factorial as described by Panse and Sukhatme (2000). The appropriate standard error of mean (S.Em. \pm) and the critical difference (C.D) were calculated at the 5 per cent level of probability.

RESULTS AND DISCUSSION

Berberis lycium seeds have poor germination percentage owing to the seed dormancy which is linked to the presence of metabolic blocks in the developed embryo or due to the presence of inhibitors in the seed coat. The results showed when the seeds treated with cow urine (T_4) and sown in a potting mixture consisting of Soil+Sand+FYM in ratio 1:1:1 (M₄) registered significantly maximum germination percent (65.33%), germination capacity (65.33%), germination energy (61.33%), mean daily germination (0.63) and peak value (1.00) shown in the Tables 1-5. Significantly better results in treatment $T_4 \times M_4$ (seeds treated with cow urine for 6 hours \times Soil+Sand+FYM in ratio 1:1:1) might be attributable to the removal of chemical inhibitors such as phenols through leaching, which made seed coat to swell and to become soft for the simple passage of water into the cotyledons. The growing medium (Soil+Sand+FYM in ratio 1:1:1) has a significant impact on the plant development. A sufficient amount of nutrients, in addition to the growing media, is essential to produce healthy seedlings with a robust root system. It is vital to choose a less expensive and nutrient rich medium that produces healthy and vigorous seedlings. Similar results discussed by Chauhan and Nautiyal (2007) in Nardostachys jatamansi.

Furthermore, immersing the seeds in cow urine leads to progressive weakening of seed coat which makes it easy for seeds to respire, absorb water and oxygen and enables the radicle to penetrate readily into the ground. Thus, imbibition of cow urine leads to greater germination per cent as well as other germination parameters. The findings are consistent with those of Garaniya and Bapodra (2015), who found that when *Abrus precatorius* seeds were soaked in cow urine for 8 hours, maximum germination was attained. Soaking of seeds in cow urine has been reported to be a good measure to enhance germination by various authors viz. Shinde and Malshe (2015) in *Manilkara hexandra*, Mukherjee and Chakraborty (2014) in *Valeriana jatamansi*, Shreesty *et al.* (2019) in *Carrisa carandas*.

Verma et al.,

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Table 1: Interaction effect of pre sowing seed treatment and potting mixture (T×M) on germination percent (%) of *Berberis lycium* under nursery condition.

Pre sowing treatment		Mean			
	M ₁	M ₂	M_3	M_4	Mean
T ₁	33.33(35.24)	36.00(36.84)	38.67(38.43)	40.00(39.20)	37.00(37.43)
T_2	38.67(38.43)	40.00(39.22)	41.33(39.94)	42.67(40.76)	40.67(39.60)
T ₃	40.00(39.20)	42.67(40.74)	41.33(39.97)	57.33(49.21)	45.33(42.28)
T_4	42.67(40.76)	45.33(42.30)	46.67(43.07)	65.33(53.99)	50.00(45.03)
Mean	38.67(38.41)	41.0(39.77)	42.00(40.36)	51.33(45.79)	
S.E.m± CD _{0.05}					
T = 1.15; $T =$	3.34				
M = 1.15; $M =$	3.34				
$T \times M = 2.31$; $T \times M$	= 6.68				
The values in parenthe	sis are transformed values (a	ngular transformation)			

 Table 2: Interaction effect of pre sowing seed treatment and potting mixture (T×M) on germination capacity

 (%) of Berberis lycium under nursery condition.

Pre sowing treatment		Maan			
	M_1	M ₂	M ₃	M_4	Mean
T ₁	33.33(35.24)	36.00(36.84)	38.67(38.43)	40.00(39.20)	37.00(37.43)
T ₂	38.67(38.43)	40.00(39.22)	41.33(39.94)	42.67(40.76)	40.67(39.60)
T ₃	40.00(39.20)	42.67(40.74)	41.33(39.97)	57.33(49.21)	45.33(42.28)
T_4	42.67(40.76)	45.33(42.30)	46.67(43.07)	65.33(53.99)	50.00(45.03)
Mean	38.67(38.41)	41.0(39.77)	42.00(40.36)	51.33(45.79)	
S.E.m± CD _{0.05}					
T = 1.15; T= 3.34					
M = 1.15; M = 3.34					
$T \times M = 2.31 \cdot T \times M = 6.6$	58				

 $T \times M = 2.31$; $T \times M = 6.68$ The values in parenthesis are transformed values (angular transformation)

Table 3: Interaction effect of pre sowing seed treatment and potting mixture (T×M) on germination energy					
(%) of <i>Berberis lycium</i> under nursery condition.					

Pre sowing treatment		Maar			
	M1	M ₂	M3	M4	Mean
T1	26.67(31.06)	32.00(34.44)	33.33(35.24)	33.33(35.24)	31.33(34.00)
T_2	33.33(35.24)	30.67(33.54)	36.00(36.85)	38.67(38.43)	34.67(36.02)
T ₃	34.67(36.00)	40.00(39.20)	38.67(38.42)	46.67(43.07)	40.00(39.17)
T_4	41.33(39.99)	42.67(40.76)	45.33(42.30)	61.33(51.55)	47.67(43.65)
Mean	34.00(35.57)	36.33(36.98)	38.33(38.20)	45.00(42.07)	
$E.m \pm CD_{0.05}$		•	•	•	•
$\Gamma = 1.01$; T = 2.93					
M = 1.01; M = 2.93					
\times M = 2.03; T \times M = 1	5.87				
he values in parenthesi	is are transformed values (a	angular transformation)			

Table 4: Interaction effect of pre sowing seed treatment and potting mixture (T×M) on mean daily germination of *Berberis lycium* under nursery condition.

Pre sowing treatment		Maan			
	M_1	M_2	M ₃	M_4	Mean
T ₁	0.32	0.35	0.37	0.38	0.36
T ₂	0.37	0.38	0.40	0.41	0.39
T ₃	0.38	0.41	0.40	0.55	0.44
T_4	0.41	0.44	0.45	0.63	0.48
Mean	0.37	0.39	0.40	0.49	
S.E.m± CD _{0.05}					
T = 0.011; T = 0.032					
M = 0.011; M = 0.032					
$T \times M = 0.022$; $T \times M = 0$.064				

 Table 5: Interaction effect of pre sowing seed treatment and potting mixture (T×M) on peak value under of

 Berberis lycium nursery condition.

Pre sowing treatment	Potting mixture				
	M_1	M_2	M ₃	M_4	Mean
T1	0.48	0.53	0.57	0.56	0.54
T ₂	0.59	0.55	0.58	0.60	0.58
T ₃	0.57	0.59	0.62	0.82	0.65
T_4	0.57	0.68	0.65	1.00	0.73
Mean	0.55	0.59	0.60	0.75	
S.E.m \pm CD _{0.05} $\Gamma = 0.03; T = 0.07$					
M = 0.03; $M = 0.07$					
$\Gamma \times M = 0.05; T \times M = 0.15$					

Verma et al.,

CONCLUSION

The seeds treated with cow urine for 6 hours (T_4) and subsequent sowing in germination media consisting of Soil+Sand+FYM in ratio 1:1:1 (M₄) proved most effective by exhibiting maximum germination percent (65.33%). The least germination percent (33.33%) was shown by control (T₁) with germination media consisting only of soil (M₁). In nursery conditions, seeds treated with cow urine for 6 hours and subsequently sown in germination media consisting of Soil+Sand+FYM in ratio 1:1:1 outperformed all other treatments for germination parameters.

FUTURE SCOPE

There is very little information available about the germination of this species on this aspect after many literature citations. Organic treatment (cow urine) helps to break the seed dormancy with improved and enhanced germination when grown on Soil+Sand+FYM (1:1:1) medium.

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