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EFFECT OF PRE-SOWING TREATMENTS ON SEED GERMINATION IN *BERBERIS ARISTATA*

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SUMMARY

The seeds of *Berberis aristata* exhibit late, erratic and poor germination potential with significant loss of germination ability. Pre-sowing chilling treatments at 5, 2 and -5^{0} C for various durations viz. 15 days, 1 month, 2 months and 3 months resulted in early and improved germination and highest (84%) germination was registered by seeds given 90 days of moist chilling at 2^{o} C. Other pre-sowing seed treatments viz. water presoaking, running water leaching and acid scarification were found very effective in induction of early germination with 40-140 per cent improvement in germination and seedling vigour.

***Key words*: Acid scarification, chilling, germination, leaching, water pre-soaking**

INTRODUCTION

Berberis aristata, commonly called as Indian Barberry, is an endangered medicinal species of Western Himalayas occurring in rare and sporadic conditions on field borders and shady ravines ranging between 1800-2800 m (Uphoff 1968, Chauhan 1999). It is rich in alkaloid Berberine, which is used in dyspepsia, diarrhoea, amenarrhoea and anorexia. However, it has not been exploited by farmers due to lack of information on propagation techniques. There is report on asexual propagation of *B. aristata* (Parmar and Khamu 1989). There is, however, dearth of information on sexual propagation of this species, though *Berberis aristata* has great potential to be propagated by seeds. Our earlier studies indicated a significant loss (20-40%) of germination ability and viability even after 6 months of short storage. Various pre-sowing treatments like chilling, osmoconditioning, priming, thermoperiodism and stratification have been shown useful in improving the germination potential and removing the climatic and physiological constraints (Seal and Gupta 1998, Wagenvoort and Opstal 1979). The present study was envisaged to evolve the methodologies to improve the germination ability and seedling vigour of *Berberis aristata*.

MATERIALS AND METHODS

Fruits of *Berberis aristata* DC were collected from the earlier marked natural populations in Khatnol (7000 feet elevation) in the months from July-October. Seeds were extracted from the freshly collected fruits, cleaned, sorted out and used for further experimentation. Uniform and healthy seeds were surface sterilized with 0.1%, $HgCl_2$ for 10 minutes. Sterilized seeds were, thereafter thoroughly washed with distilled water, surface dried and subjected to the following pre-sowing treatments.

Chilling: Two types of pre-sowing chilling treatments (dry and moist) were given to seeds. For dry chilling, seeds were kept in air tight small plastic jars and for moist

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chilling these were placed in moist substratum (double layered moist filter paper). Thereafter, both kinds of seeds were chilled at -5, 2 and 5^0 C for 15 days, 1 month, 2 months and 3 months durations.

Water presoaking: Seeds were pre-soaked in distilled water for 1, 2, 4 and 8 days at 20^oC.

Running Water Leaching (RWL): Seeds were tied in muslin cloth and placed under the fine flow of water for 8, 16 and 24 hours at 20^0 C. Thereafter, seeds were washed with distilled water and tested for germination.

Scarification: Seeds were scarified with conc. H_2SO_4 for 2, 5, 7 and 10 minutes, and thereafter washed with distilled water and tested for germination.

After subjecting seeds to above treatments, these were tested for germination and seedling vigour in dual chamber seed germinator (MAC make) by placing them on moist filter paper in Petri dishes at 20^0C temperature and 80 per cent relative humidity. The experiment was conducted in CRD with four replications of 100 seeds in each replication. Germination percentage and other attributes were tested according to ISTA (1976). The formulae used for working out the other attributes are as hereunder:

$$\text{Emergence Index (EI)} = \frac{dn}{n} \quad \text{Where dn = emergence, n = day of emergence}$$

$$\text{Germination speed} = \frac{\text{Germination percent}}{\text{Day of completion of germination}}$$

Root and shoot length were recorded 30 days after sowing of the seeds.

RESULTS AND DISCUSSION

Pre-sowing chilling (dry and moist) significantly improved germination (Table 1 and 2). Dry chilling at -5 and 2^0C for 30 and 60 days caused a substantial improvement (32-44%) in germination percentage (Table

Table 1. Effect of pre-sowing dry chilling seed treatments on germination and seedling characteristics of *Berberis aristata*

Temperature	Duration (days)	Standard germination (%)	Day of emergence	Day of completion	Emergence index	Germination speed	Root length (mm)	Seedling fresh weight (mg $seedling^{-1}$)	Seedling dry weight (mg $seedling^{-1}$)
	Control	58.18	10.00	26.67	5.82	2.23	43.36	29.87	9.90
-5^oC	15	70.00	10.33	26.00	6.81	2.70	42.50	27.90	8.63
	30	81.66	11.00	26.00	7.42	3.13	46.11	46.00	19.25
	60	83.66	12.00	25.67	7.00	4.71	42.08	37.50	14.93
	90	61.33	13.00	25.33	4.73	2.41	39.58	33.33	12.66
2^oC	15	75.67	12.00	24.33	6.64	3.11	48.33	43.33	18.10
	30	83.75	11.00	24.00	7.66	3.49	46.43	41.90	18.17
	60	77.00	11.67	23.00	6.62	3.35	41.19	36.00	14.20
	90	51.33	12.33	21.00	4.13	2.45	26.06	27.67	11.02
$+5^oC$	15	61.40	11.67	23.33	5.37	2.61	44.45	32.67	13.80
	30	68.00	11.00	20.33	6.22	3.35	45.93	35.00	13.73
	60	62.50	11.67	21.33	5.36	2.92	40.00	26.67	40.24
	90	58.34	13.00	21.00	4.48	2.76	39.49	25.17	9.61
	$CD_{0.05}$	13.57	1.52	1.49	1.66	NS	8.94	11.19	NS

1). Germination was completed up to 6 days earlier in treated seeds compared to untreated. Emergence index was significantly higher for 15 and 30 days chilled seeds at -5 and 2^0C. Maximum germination speed (4.71) was registered by seeds chilled at -5^0 C for 60 days. Seedling dry weight increased substantially i.e. 90 and 70 per cent over the control in seeds pre-chilled at -5^o C for 30 days and those pre-chilled at 2^o C for 15 and 30 days both.

Table 2 reveals that pre-sowing moist chilling was more effective than the dry chilling as it enhanced the germination and seedling growth to higher extent. Moist chilling at 2^0C for 90 days was the best treatment resulting in 84 per cent germination followed by 30 days of chilling at 2^0C and 60 days of chilling at 5^0C, which resulted in 82 per cent germination. Substantial and significant hastening in seedling emergence was recorded for all kinds of moist chilling. Likewise, completion of germination was also attained earlier in treated seeds, with the least time (7.6 days) recorded for seeds chilled at 2^0C for 30 and 60 days as compared to 10 days in control. Prominent increase in germination speed (more than 2 fold of control) was recorded for seeds chilled at 2^0C for all durations.

Thus, it is evident from the above observations that pre-sowing chilling was very effective in improving the performance of seeds. Pre-sowing chilling has been reported to be compulsory for germination in *Gentiana lutea*, a medicinal species (Yoneda *et al*. 1992). Seeds pretreated in this way show the practical independence on subsequent temperature conditions (Thompson 1969). The same is evident from the findings of the present investigation where treated seeds become capable of germinating rapidly at a higher rate and with boosted seedling vigour. Arena and Martinez (1994) have reported the enhanced germination percentage with preponement in day of emergence by 50 days of chilling in *Berberis buxifolia*. Cold treatment can promote germination by inducing GA biosynthesis (Yamaguchi *et*

Table 2. Effect of pre-sowing moist chilling seed treatments on germination and vigour characteristics of *Berberis aristata*

Temperature	Duration (days)	Standard germination (%)	Day of emergence	Day of completion	Emergence index	Germination speed	Root length (mm)	Shoot height (mm)	Seedling dry weight (mg seedling^{-1})
	Control	58.18	10.00	26.00	2.23	73.33	17.53	43.36	10.57
-5^oC	15	46.67	8.00	20.67	2.25	71.67	11.81	28.75	10.07
	30	80.00	8.33	18.67	4.28	81.67	12.17	34.17	12.67
	60	68.57	8.67	18.33	3.73	76.67	22.78	45.28	17.23
	90	30.00	9.00	18.67	1.61	70.00	34.00	35.00	10.80
2^oC	15	76.00	8.33	16.67	4.56	85.00	14.22	35.44	11.53
	30	82.00	7.67	17.33	4.71	92.67	17.83	36.67	16.47
	60	80.00	7.677	17.33	4.81	92.33	15.83	35.83	12.00
	90	84.00	7.33	17.33	4.85	93.33	19.17	43.33	16.00
$+5^oC$	15	68.50	8.67	25.33	2.70	76.67	13.89	33.11	10.83
	30	72.50	8.33	21.33	3.40	80.00	22.45	36.34	13.27
	60	82.00	8.00	23.00	3.61	81.67	19.44	35.55	9.03
	90	75.00	8.00	22.00	3.41	78.33	12.17	35.00	1.57
	$CD_{0.05}$	10.83	1.37	1.99	0.59	5.78	NS	6.65	4.45

al. 1998) or by increasing GA sensitivity (Karssen and Lacka 1986).Chilling can also have a stimulating effect on seed germination promoting factors other than GAs, such as the increase of ABA degradation (Kraepiel *et al*. 1994)..This can also be attributed to the influence of low temperature treatment on biomembrane metabolism, which plays a pivotal role in seed germination. Such treatments may instigate changes in membrane permeability (Francis and Coolbear 1987).

Pre-soaking of seeds in distilled water for various durations i.e. 1, 2, 4 and 8 days resulted in 75 to 90 per cent germination as compared to 69 per cent germination in control (Table 3). Maximum germination (90%) was obtained in seeds soaked for 1 day, followed by 80 per cent for 2 days of presoaking, thereafter, germination percentage declined. Emergence index was significantly and substantially high i.e. 10.10 for 24 hours of pre-soaking as compared to 5.69 in control. Maximum seedling vigour was registered by the seeds pre-soaked for 4 days as maximum root length (26.1 cm), shoot height (51.1. cm) and seedling dry weight (24.33 mg) were registered by seeds soaked for 8 days. Water pre-soaking enhanced germination have been reported in other species also (Caceres *et al*. 1991). *Berberis* seeds bear a thick and hard seed coat which takes longer time for imbibition. Water pre-soaking help to facilitate the imbibition which is a prerequisite for germination. In addition, it also causes the leaching of germination inhibitors.

Running Water Leaching (RWL) for 8, 16 and 24 hours was also very effective in enhancing germination (Table 4). RWL for 24 hours caused a twofold increase in germination percentage as compared to the control. Emergence and completion of germination hastened significantly, thereby shortening the germination period. Germination speed was 129 per cent higher in comparison to control as registered by seeds subjected to 16 hours of RWL followed by 120 per cent over the control for 8 hours of RWL. Similarly seedling vigour was also higher in treated seeds than the untreated ones. The maximum seedling vigour was registered by seeds subjected to 24 hours of RWL as root length, shoot height and seedling dry weight were recorded 88, 68 and 27 per cent higher than the control. Continuous flow of water during RWL may help in leaching out and washing away the inhibitory substances, thereby resulting in early induction of germination with enhanced germination percentage.

Acid scarification for 2, 5 and 7 minutes resulted in remarkable improvement in germination i.e. 96, 90 and 88 per cent, respectively compared to 40 per cent germination in unscarified seeds (Table 4). Another very important effect of acid scarification was the preponement (7-8 days) and early completion (5-13 days) of germination. Scarified seeds exhibited 3-6 times higher emergence index as compared to the control. Further, scarified seeds germinated with 192-302% higher

Table 3. Influence of water pre-soaking of seeds on germination and seedling vigour of *Berberis aristata*

Germination characters	Duration (hours)						$CD_{0.05}$
	0	24	48	96	192	288	
Standard germination	69.44	90.00	80.00	78.00	75.00	36.00	10.25
Day of emergence	12.33	9.00	12.00	19.00	13.00	16.00	3.00
Day of completion	19.33	18.66	22.00	25.00	20.66	20.00	2.00
Emergence index	5.69	10.10	6.81	4.10	5.92	2.22	1.50
Germination speed	3.61	4.91	3.82	3.12	3.65	1.77	0.69
Root length (mm)	20.00	21.66	22.33	13.33	26.11	17.33	11.13
Shoot height (mm)	43.33	28.33	53.32	28.33	51.11	33.33	5.50
Seedling dry weight (mg $seeding^{-1}$)	13.76	16.77	22.53	13.66	24.33	16.00	4..25

Table 4. Influence of other pre-sowing treatments of seeds on germination and seedling vigour of *Berberis aristata*

Germination characteristics	Control	Running water leaching duration (Hours)			Conc. H_2SO_4 (min dip)				$CD_{0.05}$
		8	16	24	2	5	7	10	
Standard germination	40.00	73.27	79.41	81.98	96.26	90.00	88.00	55.00	14.00
Day of emergence	15.33	10.00	11.00	17.33	8.33	7.33	7.00	7.00	1.021
Day of completion	30.00	25.00	26.00	26.33	25.00	19.33	16.67	17.00	3.04
Emergence index	2.61	7.40	7.28	4.72	11.60	12.26	12.87	7.85	1.62
Germination speed	1.33	2.93	3.05	3.11	3.88	4.91	5.35	3.24	0.97
Root length (mm)	16.00	27.60	28.89	30.11	33.33	31.67	58.33	20.00	2.04
Shoot height (mm)	28.00	45.53	43.67	47.11	43.33	40.00	40.00	33.33	10.00
Seedling dry weight (mg $seedling^{-1}$)	16.43	21.54	20.29	20.96	22.23	20.14	2.19	16.50	NS

germination speed than the unscarified seeds. Seedling vigour characteristics also showed substantially higher corresponding value in scarified seeds. Due to presence of very thick and hard seed coat, imbibition and consequently, seedling emergence is delayed, which can be overcome by acid scarification which dissolves the hard seed coat resulting in fast emergence of radicle (7 days earlier), which was the quickest among all types of pre-sowing treatments and resulted in early completion of germination.

Our previous studies on other medicinal species *Dioscorea deltoidea* have also indicated the significance of above pre-sowing treatments in enhancing germination and vigour (Thakur *et al.* 1997). These pre-sowing treatments probably induce an increase in germination by exploiting the seeds own resources to the maximum and providing an opportunity for low vigour seeds to cope with the more vigorous ones which may further result in obtaining higher germination percentage and vigour. Whatever the underlying exact reason is there, this is an important and desirable tract and will be of great practical implicability for propagation of this endangered medicinal species.

Based on the present findings it is concluded that pre-sowing seed treatments are of paramount importance in improving the percent germination and reducing the time for seedling emergence.

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REFERENCES

Arena, M.E. and Martinez Pastur, G. (1994). Seed propagation in *Berberis buxifolia* Lam. *Phyton Buenos - Aires*. **56:**59-63.

Caceres, A., Freire, V., Giron, I.M., Aviles, O., Pachege, G. (1991). *Moringa oleifera* (Moringaceae), ethnobotanical studies in Gutemala. *Economic Botany*. **45:**522-523. .

Chauhan, N.S. (1999). Medicinal and Aromatic Plants of Himachal Pradesh. Indus Pub. Co., New Delhi.

Francis, A. and Coolbear, P. (1987). A comparison of changes in the germination responses and phospholipid composition of naturally and artificially aged tomato seeds. *Ann. Bot*. **59:**167-72.

ISTA (1976). International rules for seed testing. *Proc. Int. Seed Test. Assoc*. **31:**1-52.

Karssen, C. M., and Lacka, E. (1986). A revision of the hormone balance theory of seed dormancy: studies on gibberellin and / or abscisic acid – deficient mutants of *Arabidopsis thaliana*. In: M. Bopp (ed.), Plant Growth Substances pp. 315-323. Springer –Verlag, Berlin.

Kraepiel, Y., Rousselin, P., Sota, B., Kerhoas, L., Einhorn, J., Caboche, M. and Miginiac, E. (1994). Analysis of phytochrome and ABA deficient mutant suggest that ABA degradation is controlled by light in *Nicotiana plumbaginifolia*. *Plant J*. **6:**665-672.

Parmar, C. and Khamu, V. (1989). A note on the sexual and asexual propagation of the Indian barberry (*Berberis aristata* DC). *Indian Forester*. **115 :** 508-509.

Seal, S. and Gupta, K. (1998). Germination behaviour of two species of Sida, a medicinal plant. *Tropical Ecology* **39:** 279-281.

Thakur, Anju, Thakur, P.S. and Bhardwaj, J. (1997). Influence of seed osmoconditioning on germination potential and seedling performance of Bell Pepper. *Seed Res*. **25 :** 25-30.

Thompson, P.A. (1969). Effect of after ripening and chilling treatments on the germination of species of Gentiana at different temperatures. *J. Hort. Sci*. **44 :** 343-358.

Uphoff, J.C. (1968). Dictionary of Economic Plants. Stechert. Henfer Service Agency Inc., New York.

Vermiere, A. (1962). Nursery and ornamental plants : A study of factors favouring propagation of plants by cuttings. *Cult. Hand*. **28 :** 650-656.

Wagenvoort, W.A. and Opstal, N.A. (1979). The effect of constant and alternating temperatures, rinsing, stratification and fertilizer on germination of some weed species. *Scientia Horti* **10:**15-20.

Yamaguchi, S., Smith, M. W., Brown, R.G.S., Kamiya, Y. and Sun T-p (1998). Phytochrome regulation and differential expression of gibberellin 3 hydroxylase genes in germinating *Arabidopsis* seeds. Plant Cell. **10:**2115-2126.

Yoneda, K., Kodama, M., Hatakeyama, Y. and Homma, N. (1992). Seed biology of medicinal plants, germination of *Gentea lutea*. *Shoykuyaco Zasshi* **46 :** 348-351.