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ENHANCEMENT OF GERMINATION IN *ABRUS PRECATORIUS L.* SEEDS BY SPECIFIC PRE-SOWING TREATMENTS

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Abstract

Herbal medicines are in great demand for preliminary health care due to their wide medicinal value, with no side effects. Since many species are used in the system of traditional medicine, scientists have great opportunities to develop appropriate packages of practices for their multiplication and conservation. Abrus precatorius is the native plant of India and used in many ways in the Indian Ayurvedic system of medicine. This seeds of the species is dormant due to hard seed coat. So the aim of the study is to remove seed dormancy and enhance germination capacity within a short period. To overcome the problem of dormancy, seeds were scarified by seed scarifier and sand paper and also treated with acid (H_2SO_4) and hot water just before sowing. 60-95% germination was achieved under different treatment conditions while the seeds without any treatment fail to germinate. The highest (95%) germination was observed just 12 days after sowing in seeds treated with concentrated H_2SO_4 for 120 minutes. 70-75% germination was achieved when the seeds were treated with acid for 105 and 135 minutes. Seeds treated with con. H_2SO_4 for 150, 90 and 60 minutes and also scarified by sand paper showed similar results with 60-65% germination. The seeds scarified by a mechanical scarifier and treated with hot water did not show more than 32.5% germination.

Keywords: *Abrus precatorius; pre-sowing; water imbibition; scarification; mechanical scarifier; germination*

Introduction

People are shifting now from modern medicines to ancient systems of medicines such as Ayurveda and others. Ancient, natural health care, tribal practices, Ayurveda, sidha and unani are the part of traditional medicine [1]. Indians have been using the Ayurvedic systems of medicine for many generations [2]. Medicinal plants are so important for health care of human beings in respect to ancient medicine system. Maximum of the traditional medicines are based on herbs, which are used by almost 80% of the world's populations.

Abrus precatorius L. (family - leguminoseae and subfamily - Papilionaceae) is a native plant of India and the East and West Indies [3], in Hindi it is known as Ratti or Gumchi. Plant parts such as leaf extracts is used for leucoderma, the seed having abrin is used as a purgative and abortive and the root extract used against coughs in the Ayurvedic system of medicine. Indian gold smiths used its seeds as weights in ancient times [4].

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The seeds of this species is dormant after shedding due to its hard seed coat so cannot germinate as per requirement without removing the hard seed coat. Without any germination strategies it's difficult to conserve the species in nature and cultivate this species to meet the demand as the cultivation of medicinal plants is only the option to fulfill the demand for the herbal drug industry and to conserve the natural resources in their natural habitat [5]. To release the pressure from wild stocks, which were exploited intensively for commercial purposes, cultivation is the only adequate approach [6]. Companies having great knowledge to control their quality and supply on the Ayurvedic medicine market in India are expanding at a rate of approximately 20% annually [7]. If required volumes and market prices are both high, cultivation can be economically feasible. So this is very important to ensure the cultivation approaches of all the medicinal plant of the demand. Keeping all these in view the present study was conducted with the seeds of *Abrus precatorius* which are very hard to germinate because of dormancy due to hard seed coat. For which seeds were treated under different treatment conditions to overcome the dormancy and enhance the germination percentage as per need.

For the endangered or threatened state of many of the plants, many factors, such as natural calamities, human settlement, road construction and unscientific exploitation, were responsible [8]. On the current market, demand is increasing, and the supply of herbal drugs is decreasing. Thus, to fulfill the gap between demand and supply, there is an urgent need to conserve and cultivate medicinally important plant species [9]. *A. precatorius* seeds exhibit potent HIV-1 PR inhibitory activity [10]. Medicinally *A. precatorius* is well reputed for its antitumor properties in Ayurvedic medicine (Indian indigenous system of medicine). Two toxic anti tumor proteins Abrin-A and B were isolated from *A. precatorius* seeds. Abrin is reported to be toxic to animals. The seed, being the potential means of propagation, needs to be extensive study for its germination requirements, at first.

Material and Methods

Mature seeds of *A. precatorius* L. were collected from naturally grown plants at the Chauras campus of Hemwati Nandan Bahuguna Garhwal University, Srinagar Garhwal, Uttarakhand, India. At the time of seed collection in the month of July, pods were split open but seeds were still firmly attached to them. Experiment was carried out from August 2011 to June 2012. Collected seeds were manually processed and undersized, compressed, discolored seeds and other debris were discarded. Seeds were carefully cleaned with tap water followed by distilled water and then used for experiment under different treatment conditions.

a. The seed scarification treatment

Seed scarifier

The seeds were scarified by a mechanical scarifier at a low speed for 5, 10 and 15 minutes and at a high speed for 2, 5 and 7 minute, separately. Only the scarified and undamaged seeds were selected for the experiment. Selected seeds were kept for germination at 25ºC in a seed germinator.

Sand paper

The seeds scratched by sand paper just to imbibe water easily and were kept for germination at 25ºC.

Hot water treatment

The seeds were placed in hot water (boiled water) until the water reached up to room temperature and then remove from the water kept for germination at 25ºC.

Acid Scarification

Fresh seeds were immersed in concentrated sulphuric acid (H_2SO_4), to observe the effect of acid on the seed coat and finally on their water imbibition and germinability. Uniformly shaped and sized seeds were divided into six lots and dipped in sulphuric acid for 60, 90, 105,

120, 135 and 150 minutes. After acid scarification, the seeds were washed thoroughly under running tap water, followed by three rinses with distilled water to remove the acid from the seeds completely. After these pre sowing treatments, seeds were kept for germination at 25ºC.

b. Imbibition

The target of seed imbibition test was to assess the accessibility of moisture to the seed. For this purpose, seeds from different treatments were taken for the imbibitions test in triplicate and each replicate comprised of 20 seeds. First the fresh weight was measured and after that the seeds were immersed in water for 24 hours at 25ºC. The imbibition was calculated by the following formula:

Water uptake % = (Weight of imbibed seeds – Initial seed weight)/Initial seed weight x 100

c. Seed Germination Test

To study germination behavior of all the seeds under different pre-sowing treatment conditions, three replicates, each containing 20 seeds, were subjected for a germination test. Seeds were placed in petridishes, lined with filter paper (Whatman No.1) and kept in a seed germinator at 25^0C. The filter paper was always kept wet with distilled water. Seeds were kept under observation till complete germination of the seeds. Radicle emergence was counted as germination of seeds. The germination percentage was calculated by using the following formula:

Germination % = (No of Germinated seeds/Total no. of seeds sown) x 100

Results

The water uptake characteristics of treated and untreated seeds are shown in Tables 1 and 2. Seeds were scarified by different methods to allow entry of water inside the seed. As a result of water imbibitions there was a significant increase in seed weight and water uptake was noticed when the seed were scarified. It is interesting to notice that the seeds without any treatment did not absorb the water due to their very hard seed coat, while scarified seeds showed imbibition of water 16-57% irrespective of the treatment conditions. Initial seed weight was 92.99mg but the seeds weight was increased upto 107.87-146.57 mg/seed (table 1 and 2). Seeds scarified by sand paper showed 133.07 mg weight and 43% water uptake after imbibitions (table 1). The water uptake of by the seeds increased as the sulphuric acid treatment time increased. Seeds treated for 60 min. with sulphuric acid showed 33.23% which was lowest while seeds treated for 150 min. showed 57.62% which was highest among the seeds treated with sulphuric acid (table 2).

Table 1. Seed weight and percent of water uptake in *A. precatorius* seeds after mechanical scarification.

Treatments	Scarification by Scarifier						Sand Paper Scarified	Hot water treatment	Control
	Low Speed (Min.)			High Speed (Min.)					
	5	10	15	2	5	7			
Weight of imbibed seed (mg) **Initial weight (92.99mg)**	108.23	116.08	118.28	107.87	115.16	120.21	133.07	115.11	92.99
Water uptake (%)	16.39	24.83	27.20	16.00	23.84	29.27	43.10	23.79	0

Table 2. Seed weight and percent of water uptake in *A. precatorius* seeds after acid treatment for different time.

Treatment Time (minute)	Control	60	90	105	120	135	150
Imbibed seed weight (mg)	92.99	123.89	126.59	128.71	139.01	143.13	146.57
Water uptake (%)	0	33.23	36.13	38.41	49.49	53.92	57.62

Seed germination and growth

Seeds were scarified by mechanical scarifier (model D094, OSAW industries) at low speed for 5, 10 and 15minutes and high speed for 2, 5 and 7 minutes. Scarification by sand paper and hot water treatment were also used to enhance germination percentage through permitting the entry of water. Under different treatments, seeds showed different germination potential. The seeds without any treatment did not show any germination. The seeds scarified by sand paper showed 65% germination. The seeds scarified at low speeds for 5, 10 and 15 min. showed 27.5, 30 and 32.5% germination respectively. The seeds scarified at high speed by the mechanical scarifier for 7 and 2 minutes, showed 35 and 27.5% germination respectively (table 3). Maximum (65%) germination percentage was recorded if the seeds were nicked with sand paper mechanically.

Table 3. Percent seed germination in *A. precatorius* L. under mechanical scarification treatment conditions.

Treatments	Scarification by Scarifier						Sand Paper Scarified	Hot water	Control
	Low Speed			High Speed					
Minute	5	10	15	2	5	7			
Germination (%)	27.5±2.5	30 ±0	32.5±2.5	27.5±2.5	30 ±5	35 ±0	65 ±5	30 ± 5	00

A. precatorius seeds treated with concentrated sulphuric acid for different times behave differently in respect to germination behaviour. The percent of germination increased as treatment time increased up to 120 minutes, but after that germination percent decreased. Highest germination (95%) was obtained in seeds treated with H_2SO_4 for 120 min followed by 105 min (75%) and 135 min (70%) after 12 days of sowing (table 4).

Table 4. Percent seed germination in *A. precatorius* L. seeds treated with sulphuric acid for different time

Treatment Time (Minute)		Control	60	90	105	120	135	150
Germination (%)	4 days after sowing	0	25	35	40	55	50	45
	8 days after sowing	0	40	55	65	70	60	60
	12 days after sowing	0	60	65	75	95	70	65

Discussion

In the present study, seeds of *Abrus precatorius* were tested for their germination potential and the shortening of their dormancy period. Therefore, it is imperative to undertake this investigation, aiming to find out the factor that can break the dormancy of *Abrus precatorius* seeds and increase its germination percentage. Seeds were subjected separately to acid scarification, mechanical scarification, sand paper scarification and hot water treatments and encouraging results were obtained which can contribute to develop its germination strategies and to conserve and cultivate the species.

Seeds treated with acids for 120 minutes showed 95% enhancement in seed germination and can be considered as the promising approach to break the dormany and germination enhancement in coparison to any other treatments. Some other workers like Alderete-Chavez et al. [11] and Nasir et al. [12] also recommended the treatment with sulphuric acid to improve the germination of *Bauhinia divaricata* L. seeds and almond seeds. The acid treatment showed that the highest germination percentage in *A. precatorius* L. seeds, treated for 120 minutes and after

that germination decreased as treatment time increased. It showed that the treatment of seeds with concentrated sulphuric acid for more than 120 minutes not only damaged the seed coat but also damaged the internal parts of the seeds (Table 4). Heidari et al. [13] also reported the effect of mechanical scarification (immersion in con. H_2SO_4) on *Prunus scoparia* seeds and observed that the stratification, along with mechanical removal of seed endocarp was more efficient than immersion in H_2SO_4. Scarification to improve germination revealed that the seed coat acted as a barrier for germination in some plant species.

The seeds scarified by sand paper showed 65% germination, while any other treatment, such as mechanical scarification at low and high speeds for different times and the hot water treatment did not show more than 32.5% germination. Sharma and Sharma [14] reported that the dormancy released effectively in *Bunium persicum* only due to moist stratification at 4^0C temperature, in contrast to H_2SO_4 scarification. Cicek and Tilki [15] applied cold stratification to enhance the seed germination percentage and germination rate in *Pterocarya fraxinifolia* (Poiret), a relic tree species. Prakash et al. [16] also reported the positive effect of stratification, growth hormones and scarification on the seed germination of some high altitude medicinal plants. A very significant effect of stratification and scarification on seed germination of *Pistacia khinjuk* stocks was observed by Baninasab and Rahemi [17] too. Alderete-Chavez et al. [18] also found a significant effect of scarification on *Lupinus leptophyllus* seeds.

The application of gibberellic acid and chilling were used to break the dormancy and enhance the germination in *Ferula asafoitida* L. by Otroshy et al. [19]. Navarro-Cano et al. [20] reported that the seed germination was significantly increased by dry heat pretreated (87 ± 3^0C for 12 min. and soaking in water at 20^0C for 48 hrs.) seeds of *Cistus heterophyllus*, a critically endangered plant in Spain. According to Qadir et al. [21] a seed soaked in water for 6 hours is ideal to support high germination.

Conclusions

It was very interesting to investigate that over the period of time the untreated seeds totally fail to uptake the water due to hard seed coat dormancy in *A. precatorius* and as a results seeds fail to germinate. Moreover, the sand paper nicking, hot water treatment and acid scarification treatment procedures applied in the present study appears applicable to break the dormancy and improve the germination potential in *A. precatorius.* Particularly pre-sowing treatment of these seeds with concentrated sulphuric acid for 120 minutes to break the seed coat dormancy and to achieve 95% germination is a promising approach.

References

[1] R. Pokharkar, R. Saraswat, V. Bhavare, M. Kanawade, *GCMS studies of Abrus precatorius*, **Pharmacologyonline**, **2**, 2011, pp. 1178-1189.

[2] S.P. Bapat, R.T. Sane, *Bio analytical studies on the process of detoxification and safety evaluation of Aconitum laciniatum and Abrus precatorius for use in ayurvedic preparation*, **International Journal of Pharmaceutical Sciences and Research**, **3**, 2012, pp. 914-921.

[3] Y.R. Chadha, **The Wealth of India, Raw Materials**, CSIR Publication, New Delhi, India, 1988, pp. 18-21.

[4] K.M. Nadkarni (editor), **Indian Materia Medica**, vol. I, Popular Prakashan, Bombay, 1976, p 5.

[5] V. Prakash, H. Bisht, P. Prasad, *Altitudinal Variation in Morpho-Physiological Attributes in Plantago major: Selection of Suitable Cultivation Site,* **Journal of Medicinal Plants Research**, 5(3), 2011, pp. 302-311.

[6] D. Lange, **Europe's Medicinal and Aromatic Plants: Their Use, Trade and Conservation**, Traffic International, Cambridge, 1998, p. 77.

[7] N. Subrat, *Ayurvedic and herbal products industry: An overview*, **Proceedings of the Workshop on Wise Practices and Experiential Learning in the Conservation and Management of Himalayan Medicinal Plants**, December 15-20, 2002, Kathmandu, Nepal.
[8] G.P. Kumar, R. Kumar, O.P. Chaurasia, *Conservation status of medicinal plants in Ladakh: Cold arid zone of Trans-Himalayas*, **Journal of Medicinal Plants Research**, **5**, 2011, pp. 685-694.
[9] N. Srivastava, V. Sharma, B. Kamal, A.K. Dobriyal, V.S. Jadon, *Advancement in research on Aconitum species (Ranunculaceae) under different area: A review*, **Biotechnology**, **9**, 2010, pp. 411-427.
[10] N.T. Marshall, **Searching for a cure: conservation of medicinal wildlife resources in East and Southern Africa**, Traffic International, Cambridge, 1998.
[11] A. Alderete-Chavez, N. de la Cruz-Landero, J.J. Guerra-Santos, E. Guevara, R. Gelabert, *Promotion of germination of Bauhinia divaricata L. seeds by effects of chemical scarification,* **Research Journal of Seed Science, 4**(1), 2011, pp. 51-57.
[12] M.A. Nasir, M.A. Summrah, A. Baksh, M.Z. Nawaz, M. Nawaz, *Effect of different scarification methods on the germination of almond nuts*, **Sarhad Journal of Agriculture**, **17**, 2001, pp. 179-182.
[13] M. Heidari, M. Rahemi, M.H. Daneshvar, *Effect of mechanical, chemical scarification and stratification on seed germination of Prunus scopariya (spach) and Prunus webbi (spach).* **American-Eurasian Journal of Agricultural & Environmental Science, 3**, 2008, pp. 114-117.
[14] R.K. Sharma, S. Sharma, *Effect of storage and cold stratification on seed physiological aspects of Bunium persicum: A threatened medicinal herb of Trans-Himalaya*, **International Journal of Botany**, **6**(2), 2010, pp. 151-156.
[15] E. Cicek, F. Tilki, *Influence of stratification on seed germination of Pterocarya fraxinifolia (Poiret) Spanch a relic tree species*, **Botany Research Journal, 3**(2), 2008, pp. 103-106.
[16] V. Prakash, H. Bisht, M.C. Nautiyal, *Seed Germination Enhancement in High Altitude Medicinal Plants of Garhwal Himalaya by Some Pre-Sowing Treatments*, **Research Journal of Seed Science**, **4**(4), 2011, 199-205.
[17] B. Baninasab, M. Rahemi, *The effects of scarification, cold stratification and Gibberellic acid treatment on germination of kholkhong seeds,* **Journal of Plant Sciences, 3**(1), 2008, pp. 121-125.
[18] A. Alderete-Chavez, D.A. Rodrignez-Trejo, E. Ojeda-Trejo, N. de la Cruz-Landero, *Effect of different scarification treatment on the germination of Lupinus leptophyllus seeds*, **International Journal of Botany**, **6**, 2010, pp. 64-68.
[19] M. Otroshy, A. Zamani, M. Khodambashi, M. Ebrahimi, P.C. Struik, *Effect of exogenous hormone and chilling on dormancy breaking of seed of asafoitida (Ferula asafoitida L.)*, **Research Journal of Seed Science, 2**, 2009, pp. 9-15.
[20] J.A. Navarro-Cano, D. Rivera, G.G. Barbera, *Induction of seed germination in Cistus heterophyllus (Cistaceae). A Rock Rose critically endangered in Spain***, Research Journal of Botany 4**(1), 2009, pp. 10-16.
[21] M. Qadir, F. Khan, S.S. Khan, *Effect of pre-treatment on the germination of Abrus precatorius Linn. by soaking in water,* **Indian Journal of Applied Pure Biol**., 27, 2012, pp. 105-108.

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