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EFFECT OF SEED SCARIFICATION ON *IN VITRO* SEED GERMINATION OF *ABRUS PRECATORIUS* L.

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Abstract

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, sand paper, hot water treatment, DMSO and different acid scarification (H_2SO_4, HNO_3) just before sowing. 50-100% germination was achieved under different treatment conditions while the seeds without any treatment fail to germinate. The highest (100%) germination was observed within 4 days at 30^0C after sowing in seeds treated with concentrated H_2SO_4 for 120 minutes. 70-85% germination was achieved when the seeds were treated with acid for 60 and 90 minutes. Seeds treated with sand paper showed similar results with 60-65% germination. The seeds scarified by a mechanical scarifier, picking with needle and treated with hot water did not show more than 25% germination after 7 days.

Key words : *Abrus precatorius*, seed germination, Dimethyl Sulfoxide (DMSO), scarification.

Introduction

World Health Organisation reports that 80% of the world's population depends on traditional medicine for their health needs. Demands of the traditional herbal medicine are increasing developing and developed countries for their non toxic in nature and having no side effect and affordable crises. According to report there was about us dollar 62 billions sales of herbal medicines in the world and it is expected to increase upto us dollar 3 trillion by 2020. Exports from India have increased in 2000 (Sing *et al.*, 2011). India is one of the mega diversity hotspots region in world one fifth of all plants found are used in medicine purpose (Schippmann *et al.*, 2002). India is also one of the largest producers of medicinal herbs and is rightly called the botanical garden of the world as it is sitting on a gold mine of well-recorded and traditionally well practiced knowledge of herbal medicine. Thus, it is necessary to developed to develop appropriate packages of practices for their multiplication and conservation which can give therapeutic activity in treatment of various diseases (Bhatia *et al.*, 2013).

Among the traditional system of medicine *Abrus precatorius* L. is one of the important herb commonly known as Indian liquorice belonging to family Fabaceae and in Hindi it is known as Ratti or Gumchi. The unique characteristic of this plant is that it has toxic red seeds with black mark at the base (Mensah, 2011). 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. The seeds of this plant exhibit potent HIV-1 PR inhibitory activity (Marshall, 1998). 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 seeds (Prakash and Nainwal, 2013). Seeds are containing several chemicals constituents and promise in treatment to several diseases (Ranjaram *et al.*, 1992 and Mohan *et al.*, 1995). Indian gold smiths used its seeds as weights in ancient times (Nadkarni, 1976).

The seeds of this plant are 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 fulfil the demand for the herbal drug industry and to conserve the natural resources in their natural habitat (Prakash *et al.*, 2011). Thus, seeds of this plant being the potential means of propagation, needs to be extensive study for its germination requirements at first.

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In the present study, seeds of *Abrus precatorius* were tested for their germination potential and shortening of dormancy period. Initial studies showed that there is very meagre percentage of germination in the seeds of this plant. Therefore, it was thought imperative to undertake this investigation to find out the factor that can break the dormancy of *Abrus precatorius* seeds and increase its germination percentage. The seeds were subjected separately to mechanical, acid scarification and pre-treatment by soaking in water.

Material and Methods

(a) Collection of plant material

Mature seeds of *A. precatorius* L. were collected from naturally grown plants at the Burdwan district, West Bengal, India. Experiment was carried out from January 2012 to June 2013 with freshly collected seeds. Seeds were carefully cleaned with tap water followed by distilled water and then used for experiment under different treatment conditions.

(b) Viability of seeds

Prior to treatment, seed viability was assessed using the chemical stain, tetrazolium chloride (International Seed Testing Association, 1999). Three replicates of 20 seeds from each collection randomly taken and were manually scarified, imbibed on plain water agar (3%) for 48 h, then transferred to a solution of 2,3,5 triphenyl tetrazolium chloride (10 g l^{-1}) for 48 h. Embryos were excised, only the embryo of seeds and most of the cotyledons stained red were deemed to be viable.

(c) Different method of seeds scarification treatment

The seeds were scarified by a mechanical method at a low speed for 5, 10 and 15 minutes and at a high speed for 2, 5 and 7 minutes, separately. Only the scarified, undamaged seeds were selected and kept for germination at 25ºC in a seed germinator. The seeds scratched by sand paper for scarification and were kept for germination at 25ºC in a seed germinator. The seeds were kept in boiled water until the water reached up to room temperature and then placed for germination at 25ºC in a seed germinator. The seeds were treated in sulphuric acid (H_2SO_4) and ntric acid (HNO_3), to observe the effect of acid on the seed coat and finally tested on their germinability. Uniform size of seeds were divided into five lots and immersed in different concentration of sulphuric acid for 30, 60, 90, 120, and 150 minutes. Then the seeds were washed thoroughly under running tap water, followed by three rinses with distilled water and kepts for germination at 25ºC in a seed germinator. Uniform size of seeds were divided into five lots and immersed in DMSO for 30, 60, 90 and 120 minutes with different concentration (5% and 10%). After scarification by this chemical the seeds were kept for germination at 25ºC in a seed germinator (Prakash and Nainwal, 2013).

(d) Seed germination test

All the seeds after different scarification treatments were subjected for a germination test in three replicates, each containing 20 seeds. Seeds were placed in petridishes, lined with filter paper (Whatman No. 1) and placed in a seed germinator at 25^0C. The filter paper was always kept wet with distilled water and observation till complete germination of the seeds. Radical 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) × 100

(e) Temperature

Temperature is an important factor for seeds germination, it affects mainly cellular metabolic and growth rates of seeds. Four temperature range *viz.* 20ºC, 25ºC, 30ºC and 35ºC were taken (in triplicate) to detect optimum temperature for seed germination of this plant.

In vitro propagation method

After germination seed were initially inoculated into 3% water agar plate for 3 days and kept in seed germinator. After that all seedling were transplanted into separate culture tube aseptically containing SNA (Seedling nutrient agar) media and incubated for suitable temperature and light condition in growth chamber.

Statistical analysis

Values are the mean ± SEM of 3 replicates. All data were subjected to students't-test analysis using SPSS soft ware package.

Results and Discussion

The plant *Abrus precatorius* L. is native in India and now grown in all tropical countries (Acharya, 2004). It is a beautiful, much-branched, slender, perennial, deciduous, woody, prickly twining or climbing herb. Seeds are elliptic to sub-globose, 0.5 cm in diameter, smooth, glossy, shining red with black blotch around the hilum (fig. A). They have a uniform weight of 1/10th of a gram, hence used as weighing unit (Tropilab, 2004) and as rosary beads by the Buddhists (Bailey, 1941).

This medicinal legume is traditionally used for medicinal purposes. Many research groups have been reported different germination rate (90% after 7 days and 95% after 12 days by using different strategies of scarification for this plant (Qadir *et al*., 2012; Prakash

Fig. 1 : A) Collected seeds of *Abrus precatorius* L., **B)** Scarified germinated seeds, **C)** *In vitro* propagation of germinated seeds, **D)** Mature plant in chamber, **E)** Different growth hormone treatment for growth of the plant, **F)** Seedling growth in pot experiment.

and Nainwal, 2013). Most of the report found that they have achieved moderate to high percentage of germination rate with longer time exposure of different chemical substances. But this report showed that 100% germination rate was achieved by concentrated sulphuric acid treatment with in 4 days, which is less time consuming process and easier method.

Initially bulk of freshly collected seeds were taken for the experiments and tested for viability determination using tetrazolium staining (table 1). The germination rate was significantly less than that predicted from the tetrazolium tests and <50% of those seeds predicted to be viable germinated (table 2). One possible reason for the lower than expected germination is that low vigour seeds, that nevertheless gave a positive tetrazolium result, succumbed to imbibitions damage during germination tests. Imbibitions injury has been reported in many species and is a particular problem in large seeded species in the *Fabaceae* (Ellis *et al.*, 1995).

The percentage of seed germination among mechanical scarification process used of sand paper (40%) method is very much effective than seed scarifier and hot water treatment process (25% and 20%, respectively) (table 1). In case of chemical scarification process different chemicals were used according to found in several literatures survey (table 2). Among different chemicals used concentrated sulphuric acid (36N) are most effective for germination (table 3). Time duration of different chemicals used are very much effective for germination of this plant. Seeds treated with concentrated sulphuric acid for 120 minutes were most effective for 100% germination (table 3). Acid scarification was found to be an effective method of breaking hard seed coat of *Abrus precatorius*. The acid treatment time required to produce an improvement in germination ranged from 30mins to 120 mins to achieve the 100% germination (fig. B). 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 3). Some other workers like Alderete-Chavez *et al*. (2011) and Nasir *et al*. (2001) also recommended the treatment with sulphuric acid to improve the germination of *Bauhinia divaricata* L. seeds and almond seeds. Acid scarification was effective at reducing fungal infection of seed, possibly leading to increased germination. The acid treatment gave the highest level of germination and it also varied between the species tested, possibly reflecting differences in seed coat thickness. Similar type of observation of immersion time varied in different

Table 1 : Determination of viability (%) by tetrazolium staining and seed germination (%) of *Abrus precatorius* L. under different mechanical condition. Control set are with out treatment. Data presented here are mean of three replicates.

Treatments	Viability (%)	Germination (%)
Control	00	00
Seed scarifier	65±1.73	25±1.15
Sand paper	84±1.15	40±1.52
Hot water	55±0.57	20±1.15

Table 2 : Determination of viability (%) by tetrazolium staining and seed germination (%) of *Abrus precatorius* L. under different chemicals treatment. Control set are with out treatment. Data presented here are mean of three replicates.

Treatments	Viability (%)	Germination (%)
Control	00	00
Conc.HNO_3 (30 minute)	65±1.73	44±2.08
Conc. H_2SO_4 (30 minute)	88±0.57	52±1.33
DMSO (30 minute)	77±1.52	40±2.08

Table 3 : Determination of seed germination (%) of *Abrus precatorius* L. under different chemical treatment condition. Control set are with out treatment. Data presented here are mean of three replicates.

Treatments	Concentrations	Germination rate (%)				
		30 min.	60 min.	90 min.	120 min.	150 min.
Nitric acid	Control	0	0	0	0	0
	3 (N)	0	0	2±0.57	4±0.57	5±1.15
	6 (N)	0	0	3±0.66	5±1.0	8±0.57
	10 (N)	2±0.57	10±1.15	12±0.57	15±1.52	22±1.15
	15 (N)	10±1.15	25±0.57	40±2.51	65±0.57	62±0.66
Sulphuric acid	Control	0	0	0	0	0
	9 (N)	2±0.57	5±1.0	12±0.57	20±0.66	22±1.15
	18 (N)	8±0.57	15±1.52	27±2.08	40±2.51	48±2.08
	27 (N)	10±1.15	24±1.73	40±2.51	50±1.52	60±0.66
	36 (N)	25±1.52	40±2.51	80±1.73	100±1.0	95±1.73
Dimethyl sulfoxide	Control	0	0	0	0	0
	5%	10±1.15	25±1.52	35±2.0	40±2.51	40±1.52
	10%	40±2.51	75±1.15	70±1.0	70±1.0	65±0.57

Table 4 : Percent seed germination in *A. precatorius* L. seeds treated with sulphuric acid for different temperature. Data presented here are mean of three replicates.

Treatment	Days	Germination (%)			
		20°C	25°C	30°C	35°C
36(N) H_2SO_4 for 120 min.	1st	0	0	0	0
	2nd	0	5±1.0	25±1.52	15±1.52
	3rd	24±0.66	30±2.08	80±1.73	65±0.57
	4th	30±2.04	55±1.0	100±0.0	80±1.73

species of acacia by Rehman *et al.* (1999), Sacheti and Al-Rawahy (1998), Teketay (1998).

Seeds from different species even seeds from the same plant germinate over a wide range of temperatures. Seeds often have a temperature range within which they will germinate and they will not do so above or below this range. Many seeds germinate at temperatures slightly above room temperature that is 16-24°C, while others germinate only in response to alternations in temperature between warm and cool. Seeds were maintained at 30°C of all the pre treatment used Sharma and Sharma (2010) reported that the dormancy released effectively in *Bunium persicum* only due to moist stratification at 4°C temperature, in contrast to H_2SO_4 scarification.

Effect of temperature for germination of this plant were also studied which showed germination rate are much more effective in 30°C for 4 days (table 4). Treatment at higher temperatures and longer durations caused a reduction in germination for most species examined (Elliot, 2000). The plant has tested for in vitro propagation with in SNA medium with trace supplement of potassium nitrate after every 7 days. The luxuriant growth and development of seedling resulted in vitro

culture and also in pot culture (figs. C, D, E and F). In this way, this plant could be conserved and also could be used for medicinal purposes.

Conclusion

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 mechanical scarification and acid scarification and encouraging results were obtained, which can contribute to develop its germination strategies and to conserve and cultivate the species.

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