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Research Article

EFFECT OF SOME PRE-SOWING SCARIFICATION TREATMENT ON WATER UPTAKE AND GERMINATION OF *ABRUS PRECATORIUS L*. (RATTI)

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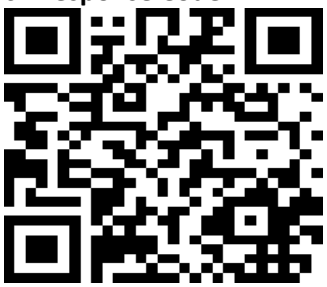

ABSTRACT

In 90 min. time duration show 26% germination within 5 days while control show 0% the present study entitled "Effect of some pre-sowing scarification treatment on water uptake and germination of Abrus precatorius L. (Fabaceae) has been carried out at seed physiology lab. Department of seed science and technology (College of Forestry and Hill Agriculture, Ranichuari Tehri Garhwal) with an aim to enhance the germination percentage of seeds of A. precatorius by breaking dormancy with different scarified methods. Seed collected local forest area, there was found that there was very less germination in A. precatorius L. in control condition, so that was need of some treatment which help to break dormancy with mechanical scarification (scarification at micropyler end, chazal end & hylum cavity.) and chemical scarification (Con H2SO4. At different time duration). The highly germination was found in chalazal end and hylum cavity Scarification (79.5 & 74.5) respectively while control show lowest (6.5%) germination and in chemical scarification with H2SO4 the maximum germination was recorded in 135 min. time duration which show 90 % germination within 5 days. And minimum germination. Root and hypocolyle length was also measured to check seedling growth. Maximum root length 4.85cm observed in 120 min soaking duration and minimum 3.40 cm in 150 min soaking duration. Water uptake was also recorded in various treated seed increasing intensity acid scarification (longer duration in acid treatment) the percentage of unlimbered seed decrease rapidly within 24 hrs of the imbibitions both hot water treated and flam exposed seed which were mechanically scarified. Heat induced water permeability of the herbs with intermittent/periodic brush fire. Seed health testing of seed borne fungal spores indicated the presence of seed born fungal spores which germinate and produce mycelium under favorable conditions. The mycelia growth becomes clearly visible on the seed surface when freshly collected seeds were incubated for even days at 250c temperature under moist conditions. It is worth mentioning that except for flame exposed seeds, in all other treatments, germinated seedlings were found to be highly impregnated fungal growth. Interestingly seed directly exposed to the flame produced rather health seedlings with very low amount of fungal mycelia growth. The results clearly indicate the seed born nature of the fungal pathogen. In acid scarification treatment the mycelia growth was no seen. This could be of great use for raising disease free seedlings of A. precatorius L.

KEY WORDS: *Abrus precatorius L,* (Ratti), pre-sowing scarification, treatment, water uptake and seed germination.

INTRODUCTION

Medicinal plant can provide a significant source of income for rural people in developing countries, especially through the sale of wild- harvested material. The collectors are often herders, shepherd or other economically marginalized sections of the population, such as landless people and women. Between 50-100% of households in the northern part of central Nepal

and about 25-50% in the middle part of same region are involved in collecting medicinal plant for sale, the materials being traded on to wholesale marked in Delhi. The money received represents 15-30% of the total income of poorer households. Medicinal plant can be symbolically very important to people. The can be held in special religious, nationalistic or ideological esteem. This can be advantageous for conservation efforts, given that it is an acknowledgement, well rooted in culture, of the worth of a sizable proportion of the world's flora. But it also carries challenges, in that this can result in dogmatic views about the medicinal properties of plants, resistance to accepting equally effective substitutes, and uncompromising attitudes towards the ownership of the plants and who should benefit from (or pay for) their continuing existence. The subject of 'medicinal plants' can arouse strong feelings, providing opportunities for bringing key conservation debates into the public arena. There is similarity to the emotions surrounding charismatic species, such as elephants and whales, with the difference that medicinal plants carry much more universal appeal. Majority of the medicinal plants being directly collected from the wilds, are facing serious threats for their long term availability. Efforts are now being made to put herbs under cultivation. In the Indian Central Himalayan hills which is yet a poor economic activity zone but a potential area with varying climatic and edaphic qualities, this prospects is being advocated at all levels. In this context, base line information on multiplication aspects becomes highly desirable. (Mayank Nutiyal., 2010). The medicinal properties of plant species have made an outstanding contribution in the origin and evolution of many traditional herbal therapies. These traditional knowledge systems have started to disappear with the passage of time due to scarcity of written documents and relatively low income in these traditions. Over the past few years, however, the medicinal plants have regained a wide recognition due to an escalating faith in herbal medicine in view of its lesser side effects compared to allopathic medicine. Through the realization of the continuous erosion of traditional knowledge of plants used for medicine in the past and the renewed interest at the present time, a need existed to revive this valuable knowledge of medicinal plants with the purpose of developing medicinal plants sectors across the different states in India. (Bhagat Singh, 2012). The species *Abrus precatorius precatorius L.* Belongs to the family Leguminosae is a native of India and West Indies (Mayank Nutiyal., 2010). It is known as Ratti or Gumchi in Hindi. Plants is used in Ayurvedic system of medicinal, like leaves extracts for leucoderma, seed abrin as purgative and abortive and root extract in cough. It has a tendency to become weedy and invasive. In India, during olden times, its seed were used as weight measure for precious metals, stones and gems. Because of their bright coloration, *A. precatorius* seeds are also used in the jewelry- making in some Asian countries. Seed are used as weights by Indian gold smiths since ancient times. Seed contains a water soluble sugar extract as D-glucose and D-mannose in 2:5 molar ratios. Preliminary investigation on the nature of constituent glucomannan (Singh *et al*., 2004a), methylation, periodate oxidation (Singh et al., 2004b), and structure elucidation of oligosaccharides (Singh and Shelley, 2003) have been reported for the parent polysaccharide structure. Present manuscript mainly deals with the isolation of the degraded glucomannan and methylation studies for proposing a possible structure of degraded *Abrus precatorius L.* seeds glucomannan.

The commercial uses of glucomannan are in the various industries linked with the food items are in sugar, textile, pudding, pastry, ice-cream industry etc. Seed glucomannan will also be explored for their air pollution minimizing capacity in the environment. Most interestingly Marshall *et al*., 1998 reported the seed of *A. precatorius* exhibiting potent HIV-1 PR inhibitory activity. did not find HIV-PR inhibitory activity in EtOH and water extracts of *A.precatorus* but had explain that contradiction in results could have been due to the differences in collcting place, collecting time and the stage of growth which could make the variation in constituent and number of compounds. It is not worthy

that HIV-1 PR is considered to be an important target for development of anti HIV-1 drugs, since this enzymes place an important role in the process of infectivity and maturation of fully infectious viron of HIV. In addition to this there are accounts of its being used as an aphrodisiac, abortifacient, having anti fertility properties, as a reliever of fever, cough, rheumatic arthritis and dysentery both in indigenous system of medicine and in the folk medicine among some tribal groups of central Himalaya. A sample of crab's eye (*A.precatorius L.*). Seed from Puerto Rico averaged 0.1088 + 0.0091 gm/seed. These seeds, which were not treated in any way, germinated at a final rate of 61 percent between 11 and 182 days after sowing. Germination is epigeous. Seed are produced in abundance. Seedlings are common in suitable habitat, but few gain access to sufficient sunlight to survive. Humans have been responsible for the long-distance transport that has resulted in the current pan tropical distribution. Birds may move the seed short distances either through curiosity or by being momentarily deceived into thinking that they are edible (personal communication with Joseph Wonderly, IITF, Rio Piedras, Puerto Rico). Lateral extension of the vines also disperses the seeds short distances. These plants can be controlled by heavy grazing, hand removal, and with herbicides. (Mayank Nutiyal., 2010).

MATERIALS AND METHODS

Seeds of *A. precatorius* L. were collected in October 20013. At the time of seed collection, the pods were split open but seeds were still firmly attached on them about 450 gm of seeds was collected and hand processed (discarding undersized, compressed and discolored seeds and other debris). The careful observation revealed occurrence of few severely infected seeds. Moreover, outer face of the pods was also seemed to be heavily impregnated with a powdery substance (fungal spores). Manual extraction of the seeds therefore may have resulted in contamination of seeds with fungal spores. Keeping this in view a seed health test was conducted. 200 seeds, contained in four replicates of 50 seeds each, were placed on 1% autoclaved agar medium. These were incubated at 25^0 C temperatures in light in a seed germinator with saturating relative humidity. Observation on mycelia growth were recorded after 7 days of incubation and the percentage of seeds sowing apparent mycelia growth was worked out. As some earlier reports and ours seeds healthy test indicated impermeability of seed coat to water, scarification of the seed coat in various ways was planned to induce water uptake as well as germination in the seeds. For water imbibitions experiment, while 100 seeds (contained in two replicates of fifty seeds each) were kept intact (seed coat not scarified), others were scarified either from micropyle end (black end) or the chalazal end (Red end). In another set of seeds the hylum cavity (a small depression on the black end of the seed) was scarified with the help of the tip of a surgical blade. In all scarification treatments, care was taken to keep damage to the cotyledons, radicle and the embryo at a minimum. Independent initial weight of five randomly selected seeds from each treatment group, including the intact seeds, was recorded. Intact seeds as well as scarified seeds were dipped in the distilled water (about three times volume of the volume of the seeds) at room temperature (10-17^0 C). Increase in the seed weight of each treatment group following imbibitions, were recorded after every eight hour interval till almost no further water intake. For the germination observation experiment, two hundred seeds contained in four replicates of fifty seeds each, under each treatment group, were platted on Whatman No. 1 filter paper (single layer) moistened with distilled water. Petri plates (9 cm. diameter) were kept at 25^0C constant temperatures in light in a seed germinator. Daily counts of germination were taken up to 20 days and the percentage of germination for each treatment group was worked out.

Acid Scarification

In addition to the mechanical scarification treatments, hot water treatments was also given to a group of seeds by dipping seeds in boiling water which cooled down on its own at the room

temperature. In another group of seeds a sort expose of seeds directly to a sprit lamp flame was given. Single seed was picked up with the help of a forceps and kept for about one second over the flame. Fresh and dried seeds were acid scarify by immersion in concentrated Sulphuric Acid (H_2SO_4) to observe the effect of acid on the seed coat and finally on the germination ability. Uniform shape and sized seeds were divided in five lots and were dipped in Sulphuric acid for 90, 105, 120, 135 and 150 min. respectively. After this treatment seeds were kept for germination at uniform temperature (25^0C) condition. Following the acid scarification, seeds were washed thoroughly under running water followed by 3 rinses with distilled water. Acid scarified seeds were also put to germinate in a germinator in the same way as described earlier.

RESULT AND DISCUSSION

Seed Health testing

The seed health testing indicated presence of seed born fungal spores which germinate and produce mycelium under favorable conditions. The mycelia growth becomes clearly visible on the seed surface when freshly collected seeds were incubated for even days at 25^0C temperature under moist conditions (Plate 1). It is worth mentioning that except for flame exposed seeds, in all other treatments, germinated seedlings were found to be highly impregnated fungal growth. Interestingly seed directly exposed to the flame produced rather health seedlings with very low amount of fungal mycelia growth. The results clearly indicate the seed born nature of the fungal pathogen. However the pathogen (spores) is generally infective. Few highly infected, deteriorated and deformed seed were also separated from the original seed lot (Plate 1). Standardization and adoption of a suitable sterilization method could be a great use for large scale production of *A. precatoius* L. seedlings. Interestingly, in acid scarification treatment the mycelial growth was not seen. This could be of great use for raising disease- free seedlings of *A. Precatoius* L.

Water uptake characteristics

The water uptake characteristics of variously treated seeds (including control) are shown in table 1 & 2. The water inhibition experiment indicated inhibition of water uptake by the seed coat. The data shown in table 2 also indicate that with increasing intensity acid scarification (longer duration in acid treatment) the percentage of unimbibed seed decreased rapidly. Therefore a need for seed coat scarification is suggestive for water uptake and the germination to commence. In the earlier experiment on seed health testing also incubated seed did not show any significant increase in the seed size even after seven days of incubation in one percent agar medium. This is probably indicative of the fact that seed coat inhibition of water uptake becomes more pronounced under water stress conditions. There was significant increase in the seed weight (as a result of water imbibitions) when the seed coat was partially scarified. Moreover, in comparison to the control, hot water treatments and direct exposure of the seeds to flame also increased water uptake by the seeds. Other than scarification a heat induced physical change in the seed coat characteristics may be the cause for increased water permeability of the seed coat. Within 24 hours of the imbibitions in both, hot water treated and flame exposed seeds imbibed almost comparable amounts of water to those seeds which were mechanically scarified. Heat induced water permeability of the seed coat and therefore resultant germination might be explanative of its invasiveness in the habitat with intermittent/ periodic bush-fires.

Seed germination and growth

The data on seed germination in *A. precatorius* L. seeds mechanically scarified (Table3) and acid scarified (Table 4) again indicate and inhibitory role of the seed coat in seed germination, although the inhibitory action does not involve any chemical inhibition or seed coat hardness. The inhibition of germination is mediated only through retraction in water uptake and probably gaseous exchange

also. The germination was lowest in control (6.5%) while it was highest in the seeds scarified from chalzal end and where hylum cavity was scarified (79.5& 74.5% respectively) (table 3). Hot water treatment and flame exposure although does not improve the germination substantially. However, a slight increase in germination may be due to the heat induced change in seed coat characteriswtics which partially allows the penetration of water. Nasev et al., (2006) suggested that almond nuts should be boiled for 10 min. before sowing to obtain maximum germination. In seeds where seeds were scarified from the micropyler end the germination increased substantially (54.5%) but not to the level when scarification is done at chalzal end or at hylum point. This could be due to the radicle injury during scarification. It is worth mentioning that according to ISTA. International rules for seed testing, it is suggested that care must be taken to scarify seed coat at a suitable part in ordered to avoid damaging the embryo and the resulting seedling. For this it is suggested that the best site for mechanical scarification is that part of the seed coat immediately above the tips of the cotyledons. In this regard, we observed a new site for scarification which is the hylum point. Scarification of *A. precatorius L*. seed at this point not only substantially increase the germination but also provide an opportunity not to damage the embryonic axis as it is located at a safe distance from the point of radical. Garcial *et al.,* (2005) reported that elimination of endocarps reduced the period of stratification needed for germination in Almond. Acid scarification is another procedure to break physical dormancy in seed of Almond (Nasir *et al.,* 2001). The acid treatment showed that the best germination percentage in *A. precatorius* L. was recorded in 135 min, while it was found lowest with increasing the tie (Table 4). The inhibitory effect of seed coat on seed germination has also been reported in Almond (Garcia-Gusano *et al.,* 2004; Nasir *et al.,* 2001; Gandio & Pedone, 1963, Garcia-Gusano *et al.,* 2005*)* and amongst other *prunus* species (Ellie *et al.,* 1985, Cetinbs & Koyuncu, 200).(Heidari *et al.,* 2008) reported the effect of mechanical scarification (immersion in con. $H2_0S0_4$) on *Prunus scoparia* seeds. They observed that the stratification, along with mechanical removal of seed endocarp is more efficient than immersion in $H2_0S0_4$.

Stone endocarp occur in all member of Prunus and sees often been thought to have seed coat doemancy and the endocarp may offer some resistance to germination (heideari *et al.,*2008; Ellie *et al.,*1985; Garicia-Gusano *et al.,* 004). Peach seeds can be removed from endocarp by applying pressure in the dorsal –vental axies (Janick & Moore, 1996).

Table 1. Percentage increases in seed fresh weight of *A. precatorius* L. following various Mechanical scarification treatments.

	R_1	R_2	R_3	Mean	SD	SE
Control	137.9	128.8	101.7	122.800	18.831	10.872
Micro.end	225.8	212.6	217.1	218.500	6.710	3.874
Chal end	216.6	214.8	235.9	222.433	11.697	6.754
Hyl. cavity	208.1	230.2	220	219.433	11.061	6.386
Flame	220.3	172.9	208	200.400	24.597	14.201
Hot water	213.8	225.8	210.9	216.833	7.900	4.561

Table 2. Percentage of unimbibed seeds previously acid treated for different time duration.

	5 Days	10 Days	15 Days	20 Days
Control	100	100	100	100
90 Min.	28	8	0	0
105 Min.	22	6	0	0
120 Min.	8	4	0	0
135 Min.	4	0	0	0
150 Min.	2	0	0	0

Table 3. Percentage seed germination of *A. precatorius L*. following various mechanical Scarification treatments.

	Control	Micro	Chal	Hyl	Flame	Hot water
	6	64	70	76	18	24
	4	46	88	80	24	40
	12	66	72	84	16	24
	4	42	88	58	30	20
Mean	6.5	54.5	79.5	74.5	22	27
SD	3.786	12.261	9.849	11.475	6.325	8.869
SE ±	2.188	7.087	5.693	6.633	3.656	5.127

Table 4. Percentage of seed germination of *A. precatorius* L. following acid treatments.

Treatment	Days after sowing			
	5 Days	10 Days	15 Days	20 Days
Control	0	0	0	0
90 Min.	26	78	98	100
105 Min.	42	92	100	100
120 Min.	68	100	100	100
135 Min.	90	100	100	100
150 Min.	66	98	100	100

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