

Full Length Research Paper

Seed treatments to break seed dormancy and Standardization of viability test procedure in *Abrus precatorius*

Pallavi, H. M.^{1*}, Vishwanath, K.², Harish, B. S.¹, Prashanth, Y.³ and Manjunath Thattimani¹

¹University of Horticultural Sciences, Bagalkot, India.

²University of Agricultural Science, Bangalore, India.

³Namdhari Seeds Pvt. Ltd., Bangalore, India.

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Abrus precatorius is an indigenous medicinal plant belongs to Fabaceae family and grow as wild vine in tropical and subtropical climate conditions. Seeds of this species possess seed dormancy and restricts germination to overcome unfavorable environmental conditions. This dormancy need to be removed to enhance germination under favourable condition of plant growth. Hence, different dormancy breaking treatments were imposed on freshly harvested seeds to improve germination. Treatments includes physical and physiological methods like soaking in water (24 h), conc. H₂SO₄ (2 min), KNO₃ (2%) (24 h), GA₃ 100 ppm (24 h), Kinetin 100 ppm (24 h) and mechanically damaging the seed coat. The experimental results revealed that *A. precatorius* possess seed dormancy, mainly due to leathery testa leading to impermeability for water and oxygen so called hard seeds. Among treatments, damaging the seed coat (Nicking) enhanced germination from 32 to 84%, followed by seeds soaked in gibberlic acid (100 pm) for 24 h (78 %). In nature, dormancy was gradually reduced and found no dormancy behavior after seven months of harvest. For quick viability test, seed coat must be mechanically damaged before preconditioning of seeds for better results. Also, seeds soaked in Tz solution of 1.0 (%) for 6 h or 0.1% for 12 h helps for clear distinguishing of viable and non viable seeds in abrus.

Key words: Abrus, medicinal plant, seed dormancy, seed viability, seed enhancement, germination.

INTRODUCTION

Seed dormancy is the resting period of seed after physiological maturity and also an adoption mechanism to overcome stress conditions. Seeds germinate when it come in contact with moisture at optimum temperature in the presence of oxygen. There are exceptional species, which does not germinate even under all favourable conditions, and this is because of the dormancy prevailing in the seeds. Majority of the medicinal plants are non domesticated, and they grow as wild plants. The wild

nature has made the seeds dormant for their survival under unfavourable conditions also this mechanism helps for their dispersal and perpetuations. Problem arises when these types of dormant species were adopted for commercial cultivation and this need to be addressed to improve the plant stand and yield.

Abrus precatorius is an indigenous medicinal plant which thrives well in tropical and subtropical climate conditions as a wild vine. It belongs to family Fabaceae and

*Corresponding author. E-mail: pallavi.hm@uhsbagalkot.edu.in

is a highly cross pollinated plant, and pollinating agents are mainly ants, bees and bugs (Ikechukwu et al., 2007). Leaves and roots of *Abrus* contain glycyrrhizin, the principal component of licorice. These tissues prepared in various ways are used to treat coughs and a number of other ailments (Parrotta, 2001). Its leaves are consumed as a vegetable and also used to prepare liquorice (Choi et al., 1989) in Central and East Africa and also used against mouth boils (chew and spit), and seed are burnt to apply on wounds (Bhagya and Sridhar, 2009). Children have been reported to suck nectar from the flowers as a snack (Jakimovich et al., 1990). Whereas, seeds are poisonous when chewed by humans and animals; however, seeds are used to simulate eyes for sculpting in Southeastern Nigeria.

Further, seeds are beautiful and uniform in size and are red colour with black spot. Because of this attractive colour birds pick them through curiosity or by being momentarily deceived into thinking that they are edible and helps in seed dispersal to short distances (Galetti, 2002). Fresh seeds are poor germinators which may be because of hard seed coat, leading to dormancy (Plate 1). The presence of hard seed coat may be impermeability to oxygen (Crocker, 1916) or mechanically resistant or inhibitors present in the seed coat or combination of these factors lead to no germination of seeds. Germination has to be improved by removing dormancy and this will helps to enhance the germ plasm under cultivation.

Livingness of any of the seed can be determined by germination and quick viability test/Tz salt test. In germination test, the dormant seeds are wrongly interpreted as ungerminated poor quality seeds or dead seeds. This disadvantage can be overcome by the Tz test by evaluating its viability. TZ test gives an early and quick snapshot of seed viability even though seeds do not germinate due to the presence of dormancy. The main principle involved here is the conversion of colourless 2,3,5-triphenyl tetrazolium chloride into stable red colour compound called 2,3,5- triphenyl farmazon, in the presence of dehydrogenase enzymes which are an indication of livingness of cells. The preconditioning of the seeds is done to activate these dehydrogenase enzymes by imbibitions process. The concentration of the Tz solution and duration of exposure influence the conversion of Tz to farmazon. Hence, there is a need to standardize the preconditioning technique of the seeds, concentration of the solution and exposure period to interpret the viability test accurately (Plate 2).

MATERIALS AND METHODS

Fresh seeds of *Abrus* were collected from Northern dry zone of Karnataka, India (16° 12' (N), 75° 45' (E), 532 m) during Rabi season. Seeds were used for the study after drying seeds to 9% moisture content. 400 seeds per treatment were treated with

different dormancy, breaking treatments in four replications. Treatments were imposed by soaking the seeds with solutions of T₁: plain water (24 h) T₂: H₂SO₄ (5 min) T₃: KNO₃ (2%) (24 h); T₄: GA₃ 100 ppm (24 h); T₅: Kinetin 100 ppm (24 h); T₆: Nicking (damaging the seed coat away from embryo); T₇: Control (untreated). Concentration of the hormones was decided based on results obtained in previous studies of dormancy. The treated seeds were air dried under shade and then tested for its germination and vigour inbetween paper method according to International Seed Testing Association (ISTA) (Annon, 1996). The germination was evaluated on the 12th day after incubation in seed growth chamber at 28°C. The hard seeds were determined based on the seeds remaining fresh, unimbibed and hard at the end of the germination test (Annon, 1996). Natural breakdown of seed dormancy was studied by storing freshly harvested seeds containing 9% moisture in polythene (200 gauge) bags under natural storage conditions. Further, stored seed was evaluated for its germination (%) and vigour at monthly intervals till seed lot recorded maximum germination (Plate 3).

Viability test

Standardization of the viability test procedure was conducted to optimize the concentration of Tz solution and period of soaking. Seeds were punctured (nicked) before soaking in water for 12 h as preconditioning treatment. At the end of the soaking period, hard seed coat became soft and was removed to expose the cotyledon. Naked cotyledon was transferred to the Tz solution of two concentration (C₁: 0.1 and C₂:0.5%) and soaked for different durations (D₁: 3 h D₂: 6 D₃: 12 and D₄: 24 h). The treatment was imposed at 30°C as enzymes are more active at this temperature. By the end of the treatment period, seeds were washed with water and evaluated by visual observation by keeping them under a dissection microscope. Cotyledon staining pattern and intensity building was observed at predetermined duration of soaking. Dissecting microscope visualized the pattern of staining and also the intensity of the colour clearly. The data obtained was analyzed for analysis of variance (ANOVA) and the means were statistically grouped by Tukey's test (Panse and Sukhatme, 1978).

RESULTS

Freshly harvested seeds showed very poor germination before any seed treatments, whereas different dormancy breaking seed treatments recorded positive response on germination because of seed treatment germination percent has increased, and decrease in hard seed percent was observed (Table 1). Germination has increased significantly from 32 to 84% after damaging the seed coat (nicking), resulting in 52% enhanced germination over control. Similarly, seeds treated with the gibberlic acid (100 ppm) for 24 h recorded germination of 78 and 15% hard seeds. Acid scarification for 2 min has also increased the germination up to 59% which was on par with 2% KNO₃ (57%) and kinetin 100 ppm (54%), whereas, the percent hard seed was 24% when scarified with acid. However, the increased duration of acid treatment resulted in an increased number of abnormal and dead seeds. Soaking of seeds in plain water for 24 h

Table 1. Influence of different dormancy breaking seed treatments on germination and seedling vigour in *Abrus precatorius*.

Treatment	Hard seed (%)	Germination (%)	Seedling length (cm)	Seedling vigour index
T ₁ : water (24 h)	35	55	12.33	678
T ₂ : H ₂ SO ₄ (2 min)	24	59	10.46	617
T ₃ : KNO ₃ (2%) (24 h)	32	57	13.16	750
T ₄ : GA ₃ 100 ppm (24 h)	15	78	12.46	971
T ₅ : Kinetin 100 ppm (24 h)	31	54	11.43	617
T ₆ : Puncturing seed coat/nicking	4	84	28.43	2388
T ₇ : Control	51	32	8.3	265
CD (0.01)	3.201	3.130	3.172	124
m±SE	1.523	1.662	1.702	1.576
CV (%)	2.95	2.62	9.46	4.53

also improved the germination significantly (55%) and reduced hard seeds (35%) over control.

Seed treatments imposed to break the seed dormancy have significantly increased the seedling length. Seeds after nicking have produced seedling of length 28.43 cm which was higher among the treatments. Short seedling was observed in untreated control (8.30 cm) followed by acid treatment (10.46 cm.). Seedlings of water soaked (12.33 cm), and growth hormones (12.46 and 11.43 cm in GA₃ and kinetin, respectively) recorded significantly longer seedlings, and are on par with each other. Seed coat nicked away from embryo recorded significantly higher seedling vigour index (2,388) followed by seeds soaked in gibberlic acid (971). Poor seedling vigour was observed in untreated controlled seeds (266) followed by acid treatment (617). The other growth hormones and water soaking treatment also significantly increased the seedling vigour over control and are on par with each other.

Natural dissipation of dormancy was studied by storing the freshly harvested seeds, and initial germination was 32% without any seed treatment. Seeds were tested for its quality in monthly intervals (Figure 2). As the storage period progressed there were decreased number hard seeds and increased germination. The germination percent increased gradually from 32 to 86% at the end of 210 days of storage, whereas hard seeds were converted to normal seeds gradually during storage of 210 days. From this study, it was observed that *Aburs* seeds lost its dormancy naturally after seven months of harvest.

Seed viability test was conducted to standardize the concentration of Tz solution and exposure period for clear distinguishing of viable and non viable seeds. Seed coat need to be punctured to facilitate the easy imbibition of water and activation of enzymes. Seed coat removed cotyledons were used soaked in different concentration of Tz solution for different periods. Irrespective of the concentration of Tz solution, staining of seeds started only

only after 3 h of soaking period. Seeds soaked in 0.1% Tz solution took complete stain after 12 h of soaking period. Whereas, seeds soaked in 1.0% took only 6 h for complete staining. Higher concentrated solution (1.0%) stained faster than the lower concentration (0.1%). At the end of the test, Tz solution of 1.0% recorded 86% of seed staining after 6 h of soaking period, which remained constant even after 24 h of soaking period. Whereas, lower concentration of 0.1% took 12 h to stain 86% of seeds tested. At the end of 24 h of soaking, seeds stained deeply in both the concentrations which made difficult in the analysis of the results (Table 2).

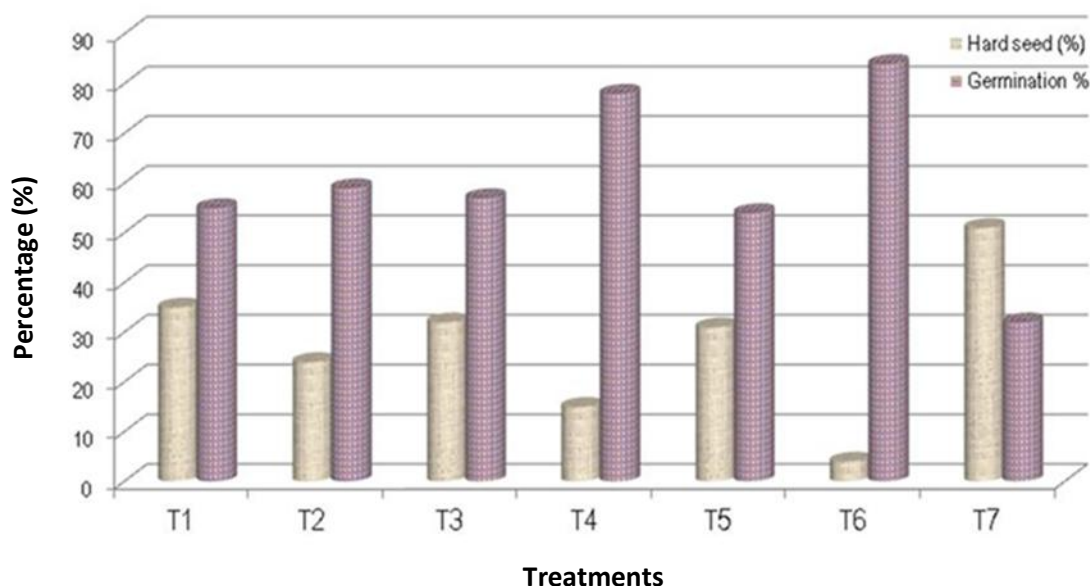
DISCUSSION

Seeds of Fabaceae family exhibit dormancy because of hard testa impermeable to water and gases (Shaik et al., 2008; Ali et al., 2011). *A. precatorius* belongs to Fabaceae family and also shows hard seeds because of leathery seed coat which hinders the absorption of water and exchange of gas (Russi et al., 1992; Lopez et al., 1999). Abrin and agglutinin-I from the seed coat are type II ribosome-inactivating proteins that inhibit protein synthesis in eukaryotic cells inhibiting imbibitions process during germination (Bagaria et al., 2006). Hard seed formation in *Abrus* as a wild climber will improve its survival in soil and allows germination only when it comes across favourable conditions and confirms its existence in nature so as to avoid extinction. Seeds produced in Northern dry zone of Karnataka (tropical climate) have re-corded 51% hard seeds may be because of dry weather prevailing during the seed maturity stages as it also influence the percent of hard seeds formed (Quinlivan and Millington, 1962).

Seed dormancy due to hard seeds is removed by puncturing the seed coat, and the germination increased to 84% (Figure 1). The damage in the leathery testa has

Table 2. Seed viability staining as influenced by concentration and duration of soaking in TZ salt solution in *Abrus precatorius*.

Duration of soaking (h)	Concentration of Tz salt solution (%)		Mean
	0.1 %	1.0 %	
3	33	65.2	49.1
6	70.6	86	77.3
12	86	88	84.7
24	88.2	88	88.1
Mean	69.45	81.3	
CD (0.01)	Concentration	Duration	Interaction
	1.584	2.239	3.169
m $\pm$ SE	3.283	-	-
Cv %	2.46	-	-

**Figure 1.** Influence of seed treatments on germination and hard seeds in *Abrus precatorius*. T₁: water (24 h); T₂: kinetin 100 ppm (24 h); T₃: KNO₃ (2%) (24 h); T₄: GA₃ 100 ppm (24 h); T₅: kinetin 100 ppm (24 h); T₆: Nicking; T₇: control.

allowed the seeds for easy imbibition of water and exchange of gases and softening of seed coat. Further, softened seed coat has allowed the growing tips to emerge out from cotyledons by pushing seed coat apart. Imbibition process initiates the physiological process of germination and results in growth of plumule and radical. Imbibition as the first phase of germination was encouraged after seed treatments and hence seeds after water soaking have increased the germination over control. This attributed to activation of enzymes, swelling and softening of seed coat (Mudasir et al., 2012) and also

leaching of germination inhibition chemicals presented in the testa (Tambat et al., 2006).

Similarly, seed treated with gibberlic acid has also increased the germination and decreased the number of hard seeds, and this is due to the activation of enzymes required for the energy generation leading to growth of the embryo. These growing tips were vigorous and hard enough to break open the seed coat and emerged as a seedling. This was also reported by Birgit et al. (2005) and proved that growth hormones release enzymes that break down carbohydrates, proteins and fats, which in

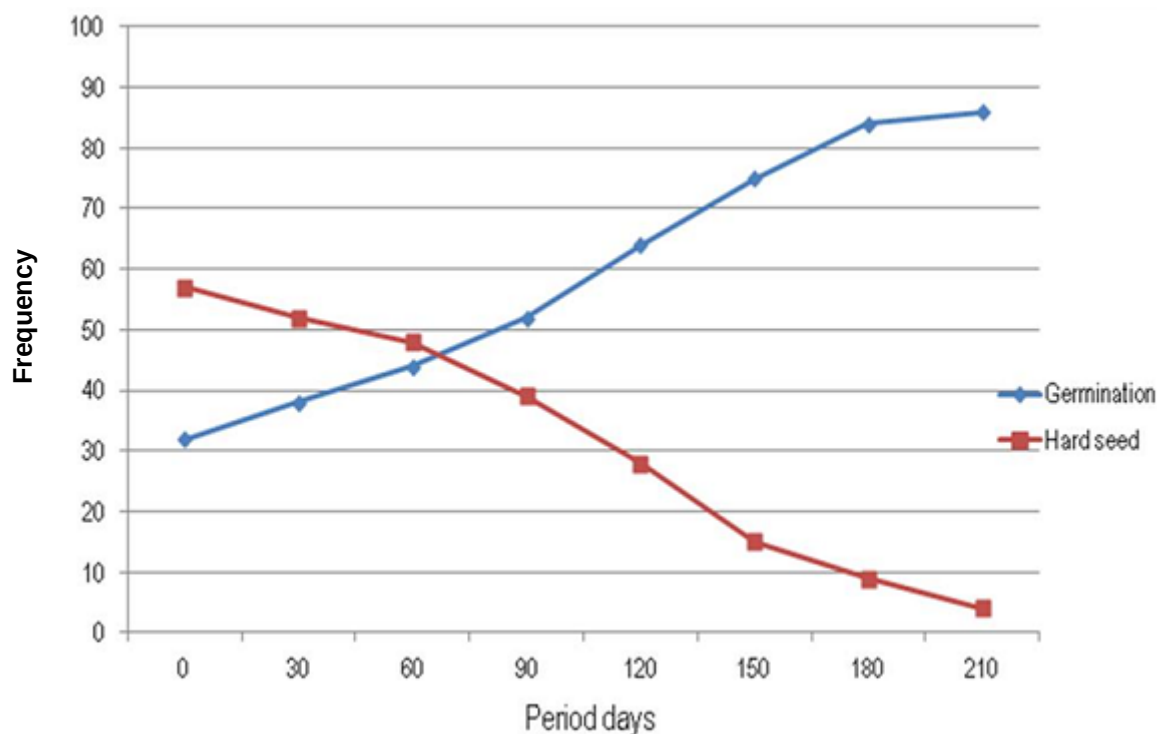


Figure 2. Germination and hard seeds under natural conditions during storage in *Abrus precatorius*.

turn release free sugars and also counteracts with inhibitors. Acid treatment is successful in reducing the hard seeds and increases the germination over control, since the acid had similar effect as that of natural degradation of the testa by microbial action in the soil (Van Staden et al., 1994).

Successful germination occurs when favourable conditions prevailed in the seed micro climate coupled with no growth inhibitors. Other seed treatments also made a positive impact on dormancy release and hence, these species are known to contain certain inhibitory compounds in seed coat and/or endosperm, which affects the germination process; external supply of growth hormones (GA_3 , kinetin, KNO_3) neutralizes such effect and facilitates germination (Evenari, 1949; Mary, 1972).

Seedling length at the end of the germination test showed significant variation among the treatments. Seeds after nicking showed higher seedling length (28.43 cm) when compared to all other treatments, seedlings showed vigours growth from the third day of the test (Plate 4). The damaged seed coat gave the way for better absorption of water, better exchange of gasses resulted in rapid growth of seedlings compared to other treatments. Seeds soaked in water, and growth hormones also noticed better seedling length than control. In untreated control, the seedling length was short (8.3 cm), this may be due to the slow rate of water

absorption which took more time for the activation of enzymes, and also the energy produced during the germination process has been utilized for break opening the leathery seed coat. Growth of the seedlings, slow, is due to the poor vigour of the dormant seeds (Mohamed et al., 1994). Seedling vigour index is also maximum in seed coat punctured seeds followed by seeds soaking in gibberlic acid. Increased germination and seedling length has contributed to the significant higher seedling vigour index, whereas, the vigour was also very poor in untreated controlled seeds.

Seed hardness of *Abrus* was naturally removed after 210 days of harvest. As the seed underwent ageing, the percentage of hard seeds decreased gradually (Figure 2). In nature, the biological cycle/rhythm will control the seed germination in order to ensure its survival in nature. Naturally, the dormancy of the seeds declines under natural conditions with time due to natural factors like disintegration of seed coat, leaching of germination inhibitors from the seed coat and microbial activities on the seed coat (Tran and Cavanagh, 1984; Van Staden et al., 1994; Mohamed et al., 1994), ultimately ensuring its survival in nature.

Concentration of the Tz solution and duration of soaking was standardized for quick viability test to know the livingness of seed. Concentration and the duration of soaking have positive relation on the seed staining pattern.



Plate 1. Germination of freshly harvested seeds before seed treatment in *Abrus precatorious*.



Plate 2. Viable and no viable abrus seeds after treating with 0.5% Tetrazolium chloride solution.

the early impact on the staining process than lower concentration. Availability of higher Tz has allowed the enzymes for conversion of Tz into farmozon in the presence of hydrogense enzymes of living seed cells. Similarly, period of soaking also influenced the staining

process and observed that increase in soaking period resulted in the uniform staining of the seeds and made easy for evaluation of the viability. Also puncturing of seed coat helps easy absorption of water and quick enzyme activation. So punctured seeds soaked in 1.0%



Plate 3. Seed germination of *abrus* after GA₃ treatment.

for 6 h showed clear staining and distinguishable pattern to identify viable and non viable seeds in *Abrus*.

Conclusion

Seed coat dormancy of *Abrus* can be overcome by making damage to the seed coat away from the embryo and it will enhance the germination. This method of dormancy breaking treatment need to be applied during seed germination test of *Abrus* also. Similarly, by soaking seeds in gibberlic acid (100 ppm) for 24 h also enhances the germination percent. Naturally, the seeds show dormancy for 210 days after which no dormancy is prevailing and ensures maximum germination. In nature, the release of dormancy is a gradual process, and complete removal of dormancy is only after seven months of seed ageing. And effective seed viability test can be conducted by soaking preconditioned punctured seeds in 1.0% Tz solution for 6 h or 0.1% Tz solution for 12 h.



Plate 4. Seed germination of *abrus* after nicking/puncturing of seed coat.

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