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EFFECT OF SEED DORMANCY BREAKING TREATMENTS ON GERMINATION BEHAVIOR OF DIFFERENT SPECIES OF MEDICINAL PLANTS OF FABACEAE FAMILY

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Abstract

Seed germination is important to know the germination pattern of a plant, more particularly the medicinal ones that might need to bring under cultivation for the primary healthcare system. The significance of the seedling in plant population ecology has long been recognized. Fabaceae family includes important medicinal plant. In this family many seeds have thick seed coat and physical dormancy. In this experiment, we chose 6 species of fabaceae family that included *Abrus precatorius* L., *Lathyrus cicera*, *Lathyrus sativus*, *Securigeria securidaca*, *Trigonella foenigraicum*, *Wisteria sinensis*. These species grow and reproduce under stress conditions; it was found that the seeds require scarification to germination. Same as other legumes, these seed eco-types have a tough seed coat, which influences germination, and susceptibility to predication. Treatment of breaking dormancy method were included, scarification (by 4 sandpaper number (60, 80, 100, 150 and 220)), Piercing seed coat (by Scalpel) and Sodium hypochlorite treatment. The results of this study contribute to an understanding of the percentage of germination and the Mean germination time of these seeds in water and scarification treatments.

Key words: Dormancy, Germination, Seed Coat, Scarification

INTRODUCTION

Legume seeds, consumed largely by populations in developing countries, are an important source of macronutrients such as proteins, carbohydrates and dietary fiber. *Securigeria securidaca*, *Lathyrus cicera* are important medicinal plant and it has high protein in seeds. *Abrus precatorius* and *Wisteria sinensis* are beautiful shrubs in china and India. One of the components of this seed is one of the most toxic substances in nature. If it reaches the blood, a few micrograms (1/1000000 g) are enough to die. It is similar to ricin (from castor oil plant) used to kill a soviet spy in London by an umbrella. *Trigonella* is important medicinal plant and use for glucose and cholesterol decreased.

The seed coat, which acts as a protective barrier for the cotyledon, has the highest concentration of phenolic compounds (Dueñas et al., 2004 and Troszynska et al., 1997). These seeds are relatively large, with a hard seed coat, able to resist mechanical damage, likewise seeds of other perennial legume species (Hyde, 1954). The impermeability of seed coats to water is typical of

many species in a number of family (e.g., Fabaceae). In the nature, there are various biotic and abiotic factors that promote seed scarification, such as mechanical abrasion of the seed coat by sand and rocks in water courses. Because of its great ability to survive under unfavorable conditions it can be considered suitable for cultivation, especially under preventive conditions for other crops. Seed germination is important to know the germination pattern of a plant, more particularly the medicinal ones that might need to bring under cultivation for the primary healthcare system. The significance of the seedling in plant population ecology has long been recognized (Zahidur Rahman et al., 2012). Many attempts have been made to investigate seed germination and seedling emergence of different annual and perennial species including medicinal plants (Chauhan and Johnson, 2008; Liebst and Schneller, 2008; Liza et al., 2010). Physical dormancy resulting from the mechanical restriction of the tegument to water uptake is the most common type of dormancy in temperate and tropical legumes. Physical dormancy is an

adaptive seed trait because it allows seeds to terminate over time and space, thus increasing the probability of their result in an adult plant. Also, hard-seeded species are able to germinate after fires, resist pathogen attack, and pass through the digestive tract of birds and mammals. Physical dormancy is overcome by scarification treatments that soften or weaken seed coverings.

Physical Dormancy Present in 15 angiosperm families Large embryos with food reserve in embryo not endosperm Hilum impermeable in *Cercis siliquastrum* Impermeable in seed coats-microphyle, hilum, chalazal area, impermeable palisade cells Embryo is not dormant Air drying during development intensifies hardness *Cytisus scoparius* – dry heat (65°C) for 2 minutes, or acid for 30 minutes Crataegus in warm climates only endocarp dormant *Robinia pseudoacacia*. The objective of this study was to assess the influence of several seed scarification methods on germination these seeds. The potential establishment of this species as a crop and the risk of its over-exploitation, with the attendant loss of biodiversity (seed is gathered from the wild and widely consumed by local people and range animals) make imperative the need of such a study, in order to optimize its seed germination and first growth.

MATERIALS AND METHODS

Seed material: Seeds were buying from the plant medicinal stores in Mashhad, and *Wisteria sinensis*

was collected directly from the nature and stored at 4 °C until their use later. Two germination experiments were carried out in completely randomized designs in high research laboratory condition at 25±2 °C temperature.

Seed weight was determined with digital scale (0.0001gram) for this work four replication with 100seeds in each ecotype differentiation and weighted. Then average this samples announcement for mean weight of 100 seeds.

Germination test: In the first experiment, the effects of simple treatments on seed germination. Four replicates of 25 seeds each were used for testing germination. The seeds were placed on the top of paper method in 9-cm Petri dishes moistened with 5 ml of distilled water. This experiment was included randomized block design. All of Petri dishes take on the try and tow dry towels were moistened with 100 ml water in trays (35×55×1.5 cm). Each tray was placed inside in sealed moisture-proof bags. After this time for keep of moisture around the samples was closed bag. Counts were made of germination daily until germination was complete or established germination after 5 days. Germination in this part was evaluated only after 21days and was kept at (25±2 °C). Water was replenished as needed.

Then seed ecotypes were differentiation to two groups: Seeds had over50% germination and under 60% germination. After that, seeds with under60% germination were differenced for other experiment.

Table 1: Characteristics of seed ecotypes and 100 seed weight (g) for each seeds.

Code of Seed Ecotype	Seed Ecotype	Production location	Production Year
A	<i>Latyris cicera</i>	Tabas	2008
B	<i>Latyris sativus</i>	Tabas	2008
C	<i>Securigeria securidaca</i>	Chenaran	2008
D	<i>Securigeria securidaca</i>	Torbat Heydarieh	2008
E	<i>Securigeria securidaca</i>	Ferdows	2008
F	<i>Trigonella foenigraeicum</i>	Tuos	2008
G	<i>Trigonella foenigraeicum</i>	Chenaran	2008
H	<i>Trigonella foenigraeicum</i>	Torogh	2008
I	<i>Abrus precatortus</i>	India	2007
J	<i>Wisteria sinensis</i>	Mashhad	2008

Scarification by sand paper and seed germination: In this part of experiment, that group of seeds had under 60% germination, Scarified by different sandpaper number. These sandpapers were included 60, 80, 100, 150 and 220. Number of sand paper was shown roughness characteristic and small number is rough and large

number is softness. After that, was counted germination when a 2mm radicle had been produced. And recorded percentage of germination and Mean Germination time (MGT) was calculated using this formula (Matthews and Khajeh-Hosseini, 2007):

$$MGT = \frac{\sum fx}{\sum f}$$

f = number of seeds newly germinated at time x ,
 x = day from when set to germination.

Piercing seed coat by Scalpel: In this part, seed coat of *Abrus precatorius* was scratched by scalpel. To achieve this, one group of seeds was mechanically scarified using a scalpel, making three approximately 2mm long incision in one side the seed coat; another group was mechanically scarified on the microphyle using a scalpel. One group of *A. precatorius* seeds not scarified and used as a control. Then seeds soaked water for 24 hours. After this time, seeds germination was counted and daily recording. Mean Germination Time and arc sin of germination percentage was calculated by Excel software.

Sodium hypochlorite treatment: In this part, Sodium hypochlorite (NaHClO₃) was used for chemical scarification. At first, Sodium hypochlorite 0.1% was prepared and *A. precatorius* seeds by distilled water for concentration. After that, was counted germination and recorded percentage of germination and Mean Germination time (MGT).

After each treatment, germination seeds were counted and recorded percentage of germination and Mean Germination time (MGT).

Statistical analysis: The data on 100 seed weight, seed germination (%) and MGT(d) were analysed

using ANOVA. The germination percentages were transformed to arc sin before analysis. The treatment means were tested by the one and two way ANOVA analysis and multiple ranges tested at the 5% level of significance. Figures were painting to Excel software and Mean comparison was performed using Minitab least-significant difference (LSD) method ($p < 0.05$). The treatment means were tested by the Duncan's multiple range test at the 5% level of significance.

RESULTS AND DISCUSSION

Comparisons of seed ecotypes, result of simple germination experiment showed that *Abrus* had very slow germination and mean germination time was more in number (MGT=12), therefore in the natural conditions, *Abrus* germination need longer time. But two ecotypes of *Latyrus* and three ecotypes of *Trigonella* had small mean germination time (MGT). Percentage of germination in this condition was above 90%. Therefore, *Latyrus* and *Trigonella* to be discount in second part of experiment. *Securigeria* seeds were shown differential response to germination to this condition. Final germination in these ecotypes was fewer than *Latyrus* and *Trigonella*, between 48% - 60% and MGT was 3.5-5.3 (day). After this time germination was stopped and other seeds were dormant seeds.

Table 2. Characteristics of seeds were clouded; 100seed weight, final germination (%) and mean germination time (d) in simple germination test.

Seed Ecotype Code	100 Seed weight (gr)	Germination (%)	MGT (d)
A	10.7	97	2.5
B	12.3	95	2.4
C	1.7	92	2.8
D	1.7	92	2.8
E	1.9	91	2.4
F	2.7	62	3.5
G	2.0	56	3.6
H	2.3	48	5.3
I	11.5	9	11.3
J	99.4	100	8.9

Germination characteristics: In Figure (2), comparison between trends of germination has been shown; first time of imbibition, rate of germination in *Abrus* seeds is zero (Figure 2(a)). 8th days after start of test germination in *Abrus* seeds was started but rate of germination is very slowly and percentage of germination after 17days is under 10%. In Figure (2-b, 2-d) trend of

germination of *Trigonella* and *Latyrus* ecotypes showed that germination was above the 90% after 3 days . Therefore, in this part *Trigonella* and *Latyrus* seeds were non-dormant and seed germination test was ended. But in figure (2-c) trend of seed germination in *Securigeria* revealed that that this ecotype germinated fewer than 60% and must be tested after scarification treatment.

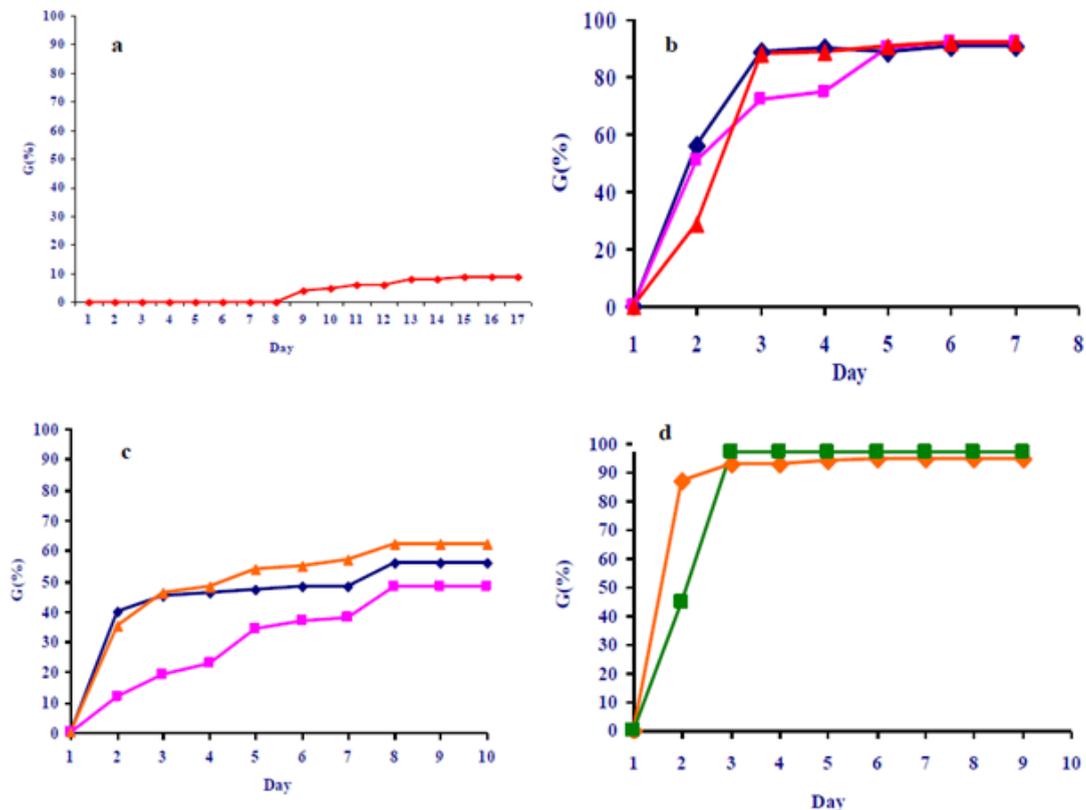


Figure 2: Trend of daily germination in 4 species of medicinal plant in this experiment.

Table3. Scarification treatment in Abrus	
Code of ecotype (treatment)	Scarification
I(1)	control
I(2)	S.D. 60
I(3)	S.D. 80
I(4)	S.D. 100
I(5)	Mic.Sc.
I(6)	Epi. Sc.

Table 4: Comparison of scarification treatment in Abrus:		
Ecotype code	Germination (%)	MGT (d)
I(1)	28.8 (31.8)a	3.000 A
I(2)	28.6 (32.3)a	8.958 A
I(3)	14.3 (18.2)ab	7.083 A
I(4)	14.3 (18.2) ab	13.83 A
I(5)	9.5 (10.8)bc	9.167 A
I(6)	4.8 (7.4)bc	3.500 A
LSD0.05	11.08	

Table 5: Final of germination (%) in <i>S. securidaca</i> , scarified by differential sandpapers number.				
Ecotype code	Control	100	150	220
C(100)	56	98	93	93
D(150)	48	85	92	93
E(220)	62	100	85	100

Ecotype code	MGT (d)	Germination (arc sin)
C(100)	6.9 ^a	82.3
D(150)	5.5 ^{ab}	73.0
E(220)	2.4 ^{bc}	80.2

Ecotype code	MGT (d)	Germination (arc sin)
J(control)	5.8 ^a	80 ^{ab}
J(60)	3.4 ^{ab}	90 ^a
J(80)	5.1 ^a	90 ^a
J(100)	4.3 ^{ab}	90 ^a

After scarified by sand papers, final germination (%) was increased and D ecotype is higher than other ecotype. In this treatment scarified by sand papers of 150 number was decreased. May be, some small seeds hasn't scarified by sand paper. Therefore this result is not showed dormant seeds after scarified.

In *W. sinensis*, mean germination time was increased after scarified and effect of scarification on final germination wasn't significant.

Response of Abrus to scarified treatment was significant and scarified mycophyle by scalpel was better than other treatment but this value was non-significant. All of treatment had negative correlation of seed weight and mean germination time was significant and negative in *S. Securidaca*. In other ecotypes had not significant correlation between MGT and seed weight.

CONCLUSION

This experiment showed that Abrus seed coat is very important reason for physical dormancy. Abrus may be had been shown for chemical materials (e.g., toxin, phenolic compound and pigments) in seed coat. Therefore, scarification by scalpel and raise of toxic compounds from seed coat showed that the best mean germination and final germination response. However, seed germination rate will increase after seed washing and seed treatment, simultaneously. It is suggested that after the scarification or piercing seed coat, the seeds to be wash daily to reduce seed coat inhibitory compounds from.

Other seed species, after suitable seed treatment had maximum speed germination and shortest germination time. However, to better investigate and close to the natural environment, this experiment must be repeated in different conditions.

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