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Breaking the Seed Dormancy by Scarification and Quantitative Analysis of Seed Germination Parameters in *Rhynchosia Suaveolens* (L.f.) D.C.

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ABSTRACT

Seed dormancy is a common problem in most of the perennial herbs for the population size increase, though it is needed in the case of most of the drought escaping plants as a part of ecological adaptability. *Rhynchosia suaveolens* (L.f.) D.C. is a perennial herbaceous plant of ethnobotanical and medicinal importance. Due to hard seed coats the plants are exhibiting seed dormancy and the scarification treatments are showing better results in the breaking of dormancy. Before performing different scarification treatments, the seeds are chemically tested for seed viability with TTC and good viability is noticed. Various seed germination parameters are calculated for each scarification treatment. Studies may help in the field of conservation biology of the species as the propagation by means of seed is a method to increase the population size.

Key words: Seed dormancy, *Rhynchosia suaveolens* (L.f.) D.C., scarification, seed viability.

INTRODUCTION

The genus *Rhynchosia* belongs to Fabaceae is having a species diversity of 22 species in our India. Some plant species of the genus are found to have bacterial nodules that enrich the soil denotes the ecological significance of these plants (Allen et al. 1981). Phytochemical studies on the members of the species revealing that these are rich in flavones, specifically glycosyl flavones (Rao et al. 1991). According to ILDIS (International Legume Database and Information Service), *Rhynchosia suaveolens* (L.f.) D.C. is the native of India and reported an extinct species in Sri Lanka. *R. suaveolens* is ethnobotanically used for treating general weakness in Seshachalam hill range of Andhra Pradesh (Sudhakar Reddy et al. 2009).

The seed is a vital stage in the life cycle of higher plant with respect to its survival as an established species. One of the most significant aspects deciding the rise of a progeny in their respective habitats is most presumably changes in dormancy levels of the seeds. The connection between the dormancy of seeds and the accomplishment of a plant as a widely propagated species is extensively established (Ali et al. 2011). The seeds differ broadly as for degree, span, and reason for dormancy. Endurance of a huge number of seeds with fluctuating dormancy degrees is the fundamental driving factor for some plant population issues each year (Ali et al. 2012). It was reported that among 260 examined *Leguminoseae* seeds, about 85% had tegument or seed coat impermeable to water. It is noted that, seed coat impermeability is associated with the presence of palisade cells. So, this kind of primary dormancy could be overcome by weakening the seed coat by allowing water to pass and that triggers germination (Cavalheiro et al. 2007). This process can also occur through the action of acids during seed digestion by zoochorous animals (Goddard et al. 2009).

Seed dormancy inferable from the existence of impenetrable layers of the seed wall cells could be discharged by debilitating the covering, along these lines permitting water to go through the layers and empower the germination of the seeds (Cavalheiro et al. 2007). Under natural conditions, the arrival of dormancy forced by the seed coat layers require the methodologies of breaking of various biological and physiological dormancy (Kelly et al. 1992).

As per the classification system for seed dormancy, there are five classes of seed dormancy were noticed viz. physiological (PD), morphological (MD), morphophysiological (MPD), physical (PY), and combinational (PY + PD). PD is expected to be caused by a physiological state of the embryo and possibly a decreased gas permeability of seed covering structures. MD is observed in seeds with embryos that are underdeveloped, especially in terms of size, but differentiated. MPD is also evident in seeds with underdeveloped embryos, but in addition they have a physiological component to their dormancy. PY is caused by water impermeable layers of palisade cells in the seed or fruit coat that control water movement. This form of dormancy is common in the members of Fabaceae family. PY + PD is evident in seeds with water-impermeable coats combined with physiological embryo dormancy (Baskin et al. 2004).

Dormant seeds which unable to grow under favourable ecological circumstances stay hard and ungerminated for a while (Koornneef et al. 2002). The coat-forced seed torpidity (hard seededness) is because of either the impermeability of the coat to water and gases, the mechanical counteraction of radicle augmentation, or the seed coat keeping inhibitory substances leaving from the undeveloped embryo or by providing inhibitors to the incipient embryo (Baskin et. al. 2000). The presence of a hard and impermeable seed coat is viewed as a boundless reason for seed dormancy in a few significant legume groups, such as, lentil, faba bean, normal bean, soybean, cowpea, Lupinus and Trifolium and that can be eradicated by scarification methods (Tiryaki et al. 2014).

Different types of mineral acids like Conc. HCl, HNO_3, and H_2SO_4 have been in use widely for eradicating seed dormancy of many species with hard seed coat, such as the species of *Astragalus* and *Medicago* (Patane et al. 2006).

The seed dormancy characteristics of *R. suaveolens*, with its hard seed coat is not known. Hence, the present study was conducted to assess the effects of mechanical (non-chemical) and chemical scarification on the release of dormancy in *R. suaveolens* seeds. Various seed germination parameters are calculated for analysis to each scarification treatment.

MATERIALS AND METHODS

Pods of *R. suaveolens* are collected‘ from the forest area of Seshachalam hill range of Tirumala and separated seeds. The seeds are stored in sealed bags for further use. Healthy and similar type of seeds are selected for the experimentation. The mature and dry pods of *R. suaveolens* exhibiting explosive dehiscence to eject the seed with bi-valvate configuration. The seeds are shiny, striate, reniform and pigmented with greyish brown or black appearance and measures 4.3 ± 0.3 mm long, 3.2 ± 0.3 mm wide strophiolate.

Chemical seed viability testing

The TTC Test (Tetrazolium test) originally developed by Lakon (1949) used to assess seed viability, prior to scarification treatments performed with the application of TTC (2,3,5-tryphenyl tetrazolium chloride) on cut single seeds as a rapid method. The test relies on the reduction of the colourless and water soluble TTC to an insoluble red Formazan compound. This reduction occurs as a result of hydrogen ions donated to the TTC upon dehydrogenase activity in metabolically active embryonic tissues.

Consequently, seed viability is usually determined by a visual observation of finding the characteristic pattern and intensity of formazan staining pattern for individual seed embryos. 1% aqueous solution of TTC is prepared and the soaked seeds are placed after removing the seed coats and making the seeds into two parts along the cleavage of the cotyledons (Verma et al. 2013). The total set up is left for the incubation of time period about 24 hrs. in the dark condition so as to prohibit the photooxidation.

Scarification treatments

Seeds are taken in 25 number in total and 5 seeds for each petri plate to perform each scarification test. Scarification treatments include the physical scarification with the sand paper and chemical scarification, includes the treatment of seeds with Concentrated acids H_2SO_4, HCl and HNO_3. The chemical scarification methods are adopted for a standard time period of 25 minutes. Seeds are kept in hot water of 70^0 C and left the container at the room temperature allowing it for cooling. It is one of the non-chemical alternatives used for scarification of seeds.

After the treatments, the seeds are placed on wetted filter papers in petri plates and kept for observation for 15 days in the laboratory at room temperature and humidity conditions. The results are noted every day and the data is analyzed for various germination parameters statistically by One-way ANOVA of SSS online package.

Seed germination parameters

Six different parameters related to seed germination were assessed according to the standard formulae. Final germination percentage (G), mean germination time (MGT), coefficient of velocity of germination (CVG) and germination rate index (GRI) were estimated as suggested by Al Mudaris (1998) and mean germination rate (MGR) following the method suggested by Ranal et al. (2009). Timson's germination index was calculated using the formula reported by Khan et al. (1984).

RESULTS AND DISCUSSION

Seed viability test turned the cotyledons into frozen red colouration after the incubation period, giving the inference that the selected seeds are 98% (±2.739) viable. This indicates that the seeds are viable and having the capacity to germinate but the problem is seed dormancy conferred by the impermeable seed coat.

Many species of the Fabaceae, such as *Lupinus* species seeds exhibit dormancy that is primarily due to water impermeability of the outer seed coat. Seed dormancy, an apparently inert or resting state of the seed, stops seed germination of plant species over a prolonged stretch of time periods. Seeds with dormancy permit the plants to overcome the unfavorable conditions (Ali et al. 2011). Scarification of *L. texensis* seeds with conc. Sulfuric acid for 30 to 60 min improved seedling emergence. It was reported that the acid scarification did not reduce thickness or strength of the seed coat, but created several small pores in the seed coat which likely facilitated imbibition. The experimental results showing that seed exhibits Physical dormancy conferred by the impermeability of seed coat, that is making the seed to be remain in dormant condition. The values of G% and T50 denoting that, the conc. H_2SO_4 treatment is found to be the best method out of all treatments for the eradication of the dormancy of seeds in *R. suaveolens*. Similar value of G% is attained in the physical scarification method also but T50 is high denoting the delayed response for the treatment. Hot water treatment is also promoting seed germination but it is not as much as the Conc. H_2SO_4 treatment and Physical scarification. The scarification with HCl and HNO_3 are found to be negative effect in the results showing no effect on the seed coat permeability (Table 1).

Seed germination is the main process that triggers the growth of the seedlings that are responsible for the increase in the population of the species. *Rhynchosia suaveolens* seeds are hard and impermeable, scarification is suggestable method for the removal of dormancy as the process increases the permeability by weakening the seed wall layers. Mechanical scarification and treatment with Conc. H_2SO_4 are found to be the best methods. There are certain inferences we can made from the measured seed germination parameters. With the increase of the FGP values increase in the seed germination can be observed (Scott et al. 1984). The lower values of MGT indicate the faster germination (Orchard 1977). The CVG indicates the speedy germination of the seeds. If the value increases the required time for

Table 1. Germination of *R. suaveolens* seeds under various scarification treatments

Treatment	G%FGP	T50 Days	MGT Days	MGR	CV	GRI	TGI
H_2SO_4	98.4±2.19	2.6±0.22	3.68±0.1	0.27±0.007	27.18±0.79	27.59±1.01	24.6±0.54
HCl	0	0	0	0	0	0	0
HNO_3	0	0	0	0	0	0	0
HOT WATER	81.6±2.19	3.2±0.27	4.85±0.13	0.20±0.005	20.63±0.27	20.63±0.57	16.93±0.54
PHYSICAL	98.4±219	3.7±0.27	4.68±0.1	0.21±0.004	21.37±0.49	21.37±0.49	16.39±0.36

G% : Final Germination Percentage (FGP); MGT: Mean Germination Time; MGR: Mean Germination Rate; CV: Coefficient of Velocity of Germination; GRI: Germination Rate Index; TGI: Timson's Germination Index the germination process decreases. The highest possible value of CVG is 100 which can be attained if the total germination of the selected sample takes place on the first day itself (Jones et al. 1987). The GRI value stands for the germination percentage on each day during the germination span. High GRI indicates the high and fast germination. Probable natural release of physical dormancy is seeds to be exposed to the specific environmental conditions. For some species of Fabaceae, moist conditions in the environment are required for the breaking of Physical dormancy (Rodrigues-Junior et al. 2018).

Abrasing with sandpaper is reported as a very effective method in breaking seed dormancy in *Rhynchosia capitata*, and germination to 100% as compared to HCl treatments. The probable mechanism of increase in seed germination influenced by Conc. H_2SO_4 is due to its capability to weakening the seed coat that leads to water intake and seed imbibition (Ali et al. 2011). As per the reports available, in case of forage legume tree species, the most efficient seed dormancy eradication method was immersion in Conc. H_2SO_4, while in the case of forage herbaceous legumes, the most efficient seed dormancy eradication treatments were immersion in concentrated sulfuric acid, sandpapering (physical or mechanical method) and hot water. In general, the scarification methods signified with the positive results in dormancy eradication resulted in significantly higher total germination percentages. As in the case of present study, the other relevant works associated with the some of the examples are, the use of immersion of seeds in H_2SO_4 for five minutes of *Neonotonia wightii* and *Pueraria phaseoloides* was reported as the most suitable method of dormancy removal and scarification with sandpaper stands good with the dormancy removal of the seeds of *Calopogonium mucunoides* (Morais et al. 2014). Sulfuric acid scarification generates many cracks and cavities in the seed coat that may help in water absorption and O_2 diffusion required for the embryo. Increased water absorption is known to overcome seed dormancy in many tree species (Tanaka-Oda et al. 2009). Similarly, the results of the experiments conducted on *R. capitata* seeds showed that Mechanical scarification was the best method to overcome this coat-imposed dormancy, apart from softening of the seed coat by soaking in conc. H_2SO_4, HCl, and HNO_3 that significantly increased seed germination (Ali et al. 2011).

Considering the results of the present study, it is possible that the mechanical forces of seeds and sand may be weakening of seed coat. Better results of scarification with HCl and HNO_3 in *R. capitata* (Morais et al. 2014) might be due to the prolonged leaching treatment and the varied composition of seed coat material when compared to *R. suaveolens*. No remarkable results are observed in the present study for the scarification treatments with HCl and HNO_3 may be due to the scarification for short time period.

CONCLUSIONS

In general, the results of these experiments showed that seeds of *R. suaveolens* exhibited hard seed coat dormancy. Softening the seed coat by soaking the seeds in H_2SO_4 significantly increased seed germination. Mechanical scarification was found to be the best nonchemical treatment to overcome this coat-imposed dormancy in the seeds of this species. It can be concluded that the seed coat was the major barrier to *R. suaveolens* seed germination that imparts dormancy to seed.

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