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Evaluation of different pre-sowing seed treatments to break dormancy of *Crotalaria senegalensis*, a famous rangeland forage in Sudan

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ABSTRACT

The aim of the present investigation was to evaluate various methods for breaking dormancy of Crotalaria senegalensis seeds. The seeds were subjected to the following treatments for physical and physiological dormancy breaks: (1) physical scarification which carried out by soaking intact seeds in distilled water for 24 h at ambient temperature (25°C) , soaking in hot distilled water (80°C) for 15 and 30 min, immersion in H_2SO_4 (98%) for 5, 15 and 30 min and mechanical scarification by sand paper. The physiological dormancy break treatments include: (1) soaking scarified seeds in distilled water for 24 h at ambient temperature (25°C), (2) immersion in 1%, 2% and 4% KNO_3 and (3) soaking in golden leaf purslane extract for 8, 16 and 24 h. All scarification treatments improved the germination capacity of C. senegalensis seeds, the highest being after soaking in golden leaf extract followed by soaking scarified seeds in water for 24 h and immersion in 1% KNO_3. The results showed that using mechanical scarification with sand paper and immersion seeds in H_2SO_4 (98%) for 30 min to break seed physical dormancy of C. senegalensis was the most effective treatments but inefficient because mechanical scarification break small seeds of the crotalaria especially for large quantities lots and it was proven to be a labor intensive process. On the other hand the acid H_2SO_4 treatment is costly and dangerous in handling. To break physiological dormancy of C. senegalensis seeds, soaking in golden leaf purslane extract for 16 h represent the most recommended treatment.

Keywords: Crotalaria senegalensis, Germination rate, Physical dormancy, Sandy paper scarification.

INTRODUCTION

Cultivation of forage legumes in rangelands and pastures has become a technique of growing use by farmers, because it is a sustainable way to recover degraded pastures, and at the same time provide quality forage for animals. Rangeland productivity may be increased by introducing the nitrogen-fixing legumes into the rangelands [1] [2] [3]. Crotalaria is a large genus comprising of more than 600 species scattered all over the tropics and sub-tropics [4]. This genus shows largest species diversity in tropical Africa followed by Southeast Asia and Central America [5]. In Sudan, there are two species, *Crotalaria senegalensis* and *Crotalaria retusa*, locally called "Safari", both grow in areas degraded by erosion on infertile soils and of bad physical properties. The plant is a popular forage shrub due to its high crude protein content (about 17%) and an excellent palatability for grazing animals such as camels, cows and goats in natural ranges of Sudan. *C. senegalensis* (Safari) has an adaptive advantage of having an annual cycle combined with a "seed escape" habit. The plant as a self-reseeding legume has developed specific strategies to ensure adaptation and reproduction under harsh climatic conditions [4].

Generally, legumes seeds exhibit hardseededness resulting in dormancy [6]. Several studies have been conducted on legume germination using different seed coat pre-sowing treatments [7] [8] [9]. The function of the seed coat is to protect the embryo and endosperm from desiccation, mechanical injury, unfavorable temperatures and attacks by bacteria, fungi and insects [10]. Similarly to many other taxa of legumes, the seeds of Crotalaria plants remain in a state of physical dormancy until the seed coat is made permeable by some environmental factors in natural conditions [11].

In wild species, physical dormancy release could not be only the effects of the gastric juice of birds and rodents that eat the seeds, but it could also be influenced by the partial chewing and high temperatures and humidity to which the seeds are exposed when are in contact to feces [12] [13].

This research aims at evaluating the impact of various pre-sowing seed treatments on the germination response of *Crotalaria senegalensis* and at developing an effective method of breaking seed dormancy. The study is crucial for optimizing seed germination and growth of Crotalaria since the increasing demand for fresh materials as a promising forage plant in vulnerable and fragile natural range in Sudan which could result in the attrition of natural populations to the point of extinction and a resultant loss of biodiversity.

MATERIALS AND METHODS

Pre-sowing seed treatments

Crotalaria senegalensis seeds were obtained from Sudanese Administration of Forage and Range, Ministry of Animal resources and Fisheries in Gadarif State in April 2015. The fresh weight of 1000 seeds was 7.0 g and the moisture content was 4.6% on dry weigh basis, which was estimated after drying the seeds at 70 ^{o}C for 24h. All seeds were surface sterilized in sodium hypochlorite (1% NaOCl) for 3 minutes and then washed three times with sterile distilled water prior to an experimental procedure to prevent fungal contamination. Seeds were subjected to different mechanical, physical, chemical and biological treatments. Mechanical scarification was achieved by vigorously rubbing the seeds for 10 sec between two sheets of fine-grained sand paper to remove the testa without injuring the embryo. Physical scarification was carried out by soaking intact seeds in distilled water for 24 h at ambient temperature (25°C) and in hot distilled water (80°C) for 15 and 30 min. After completion of hot water treatments, seeds were removed from the water and left to cool for 10 min. Samples of mechanical scarified seeds were soaked in distilled water for 24 h at ambient temperature (25°C) and Chemical scarification was accomplished using two different techniques. First, samples of intact seeds were soaked separately in concentrated sulphuric acid (98% H_2SO_4) for 5, 15 and 30 min. Second, samples of mechanically scarified seeds soaked separately in potassium nitrate (KNO_3) at 0, 1, 2 and 4 % for 24 h. Biological treatments was carried out by soaking scarified seeds in extract of golden leaf purslane (*Portulaca oleraceae* L.) for 8, 16, 24 h. All seeds in the H_2SO_4, KNO_3 and extract of golden leaf purslane treatments

were thoroughly washed three times in sterile distilled water before culturing. Intact seeds without pre-sowing treatments were considered as the control.

Germination tests

The germination percentages is an estimate of the viability of seeds. Germinated seeds were counted every 48 h for 20 days. In the present study, Seeds were placed in sterile plastic Petri dishes (12.5 cm), containing moistened filter paper. All Petri dishes were incubated for 20 days at 25°C and 16 h photoperiod by a fluorescent light at 40 µmol m^{-2} s^{-1}. According to [14]Sharma and Sharma (2010), seeds were considered germinated upon emergence of radicals (≥ 2 mm). The following germination parameters were recorded:

1)Final germination percentage (FGP) = (number of number of germinated seeds/number of total seeds) X100
2)Mean time to germination (MTG or G_{50}) was calculated according to the following equation [15] (Moradi *et al*., 2008).

MTG or $G_{50} = \sum Dn / \sum n$

Where,
n = number of seeds which were germinated on day D.
D = the number of days counted from the beginning of germination.

3)Germination rate index (GRI)

GRI = [G1/1 + G2/2 + ……..+ Gx/x]

Where,
G = the germination on each alternative day after placement.
1, 2, x = the corresponding day of germination [16] (Esechie, 1994)

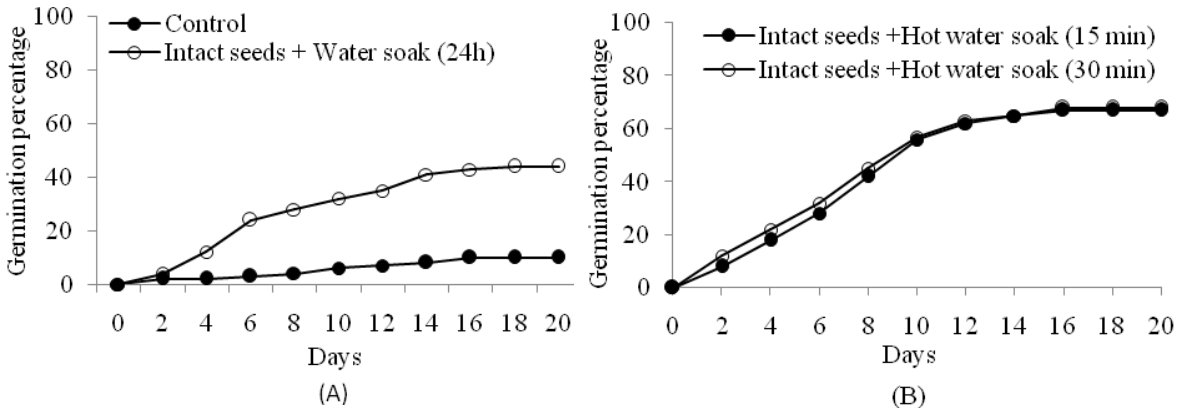
4)Corrected germination rate index (CGRI)

CGRI = (GRI/FGP) X 100

Experimental design and statistical analysis
All experiments were conducted in a completely randomized design. There were 15 treatments replicated 4 times, and each replication consisted of 10 seeds. Data were subjected to one way analysis of variance (ANOVA) appropriate for a completely randomized design and mean separation among treatments was carried out by Least Significant Difference (LSD) using SPSS program (version 15). Excel computer software was used for making graphs.

RESULTS AND DISCUSSION

The effects of various pre-sowing seed treatments on the time-course changes in germination percentage of Crotalaria senegalensis are shown in **(Fig.1)**. The germination percentage GP for the control was significantly ($P < 0.05$) lower than the GP recorded for intact seeds soaked in water for 24 h at ambient temperature at all germination reading occasions **(Fig. 1A)**. In the hot water treatment soaking treatments, germination percentage at both exposure time to hot water was identical **(Fig. 1B)**. Germination percentage in both 15 and 30 min hot water treatments were better than the control and soaking in water at ambient temperature treatments. In the H_2SO_4 treatments, germination percentage was improved with increasing exposure time to the acid **(Fig.1C)**. Mechanical scarification of intact seeds significantly ($P < 0.05$) increased germination percentage and recorded the highest germination percentage among all treatments during the entire germination period followed by immersion of intact seeds in H_2SO_4 for 30 min treatments. Mechanical scarified seeds and scarified seeds soaked in H_2O for 24h were identical during the time- course of germination **(Fig. 1D)**. Immersion scarified seeds in KNO_3 significantly increased germination percentage compared with control. Interestingly immersion of seeds in 1% KNO_3 significantly ($P < 0.05$) increased germination percentage and recorded the highest GP among KNO_3 treatments during the course of Crotalaria seeds germination at all occasions **(Fig. 1E)**. Scarified seeds soaked in golden leaf purslane extract for 16 h significantly increased germination rate compared with the soaking in purslane in 8 and 24 h through the entire time-course of germination **(Fig. 1 F)**.



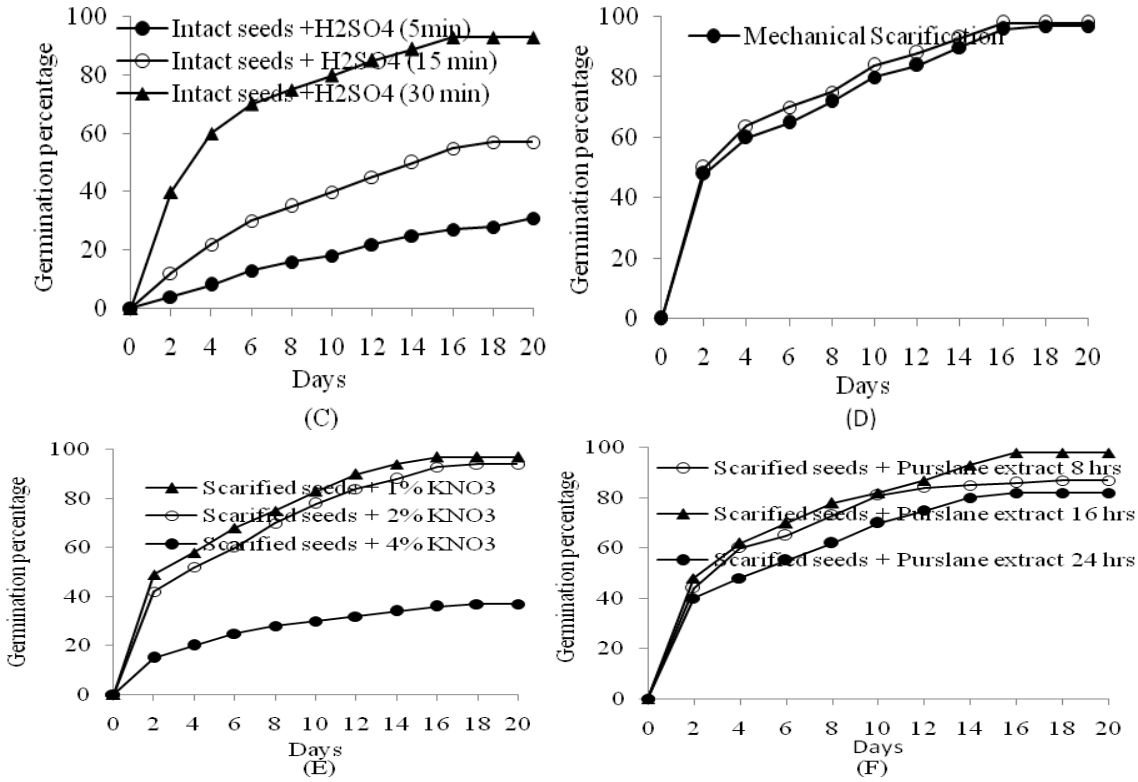


Fig 1. Time-course changes in germination percentage of Crotalaria senegalensis seeds as affected by different pre-sowing treatments (intact seeds (A, B, C), scarified seeds (D, E, F)) over 20 days

Table 1: Effect of pre-sowing treatments on final germination percentage (FGP) germination rate index (GRI), corrected germination index (CGRI) and time taken to reach 50% of final germination percentage (GT_{50}) for *Crotalaria senegalensis* after 20 days in culture

Treatments	FGP	GRI	CGRI	GT_{50}
Intact seeds				
Control	9.5 (17.9) i	1.5 i	15.7 f	3.8 e
Water soak (24h)	43.8 (41.4) f	10.0 g	22.8 e	5.0 c
Hot water soak (15 min)	67.3 (55.1) e	20.8 e	30.9 c	6.5 a
Hot water soak (30 min)	67.5 (55.3) e	21.0 e	31.1 c	5.8 b
H_2SO_4 (5 min)	30.5 (33.5) h	7.8 h	27.5 d	7.0 a
H_2SO_4 (15 min)	56.5 (48.8) g	18.5 f	32.7 b	5.5 b
H_2SO_4 (30 min)	92.3 (74.5) b	35.5 b	38.4 a	2.5 f
Mechanical Scarification	98.0 (83.0) a	38.3 a	39.1 a	2.0 f
Scarified seeds				
Water soak (24h)	97.8 (82.6) a	38.0 a	38.9 a	2.0 f
1% KNO_3	97.0 (80.4) a	37.2 a	38.3 a	2.0 f
2 % KNO_3	93.5 (75.3) b	36.0 b	38.5 a	2.6 f
4 % KNO_3	37.0 (37.5) f	8.2 h	22.2 e	3.8d
Purslane extract soak (8 h)	87.3 (69.1) c	30.2 c	34.6 b	2.0 f
Purslane extract soak (16 h)	98.5 (84.0) a	38.5 a	39.0 a	2.2 f
Purslane extract soak (24 h)	82.0 (64.9) d	28.2 d	34.4 b	2.2 f
$LSD_{0.05}$	4.2	1.6	2.3	0.6

Effects of pre-sowing treatments on final germination percentage (FGP), germination rate index (GRI), corrected germination percentage (CGP) and time to 50% of germination (GT_{50}) were shown in Table 1. All breaking dormancy treatments significantly ($P < 0.05$) affected germination attributes of C. senegalensis. In treatments using intact seeds, mechanical scarification with sand paper significantly ($P < 0.05$) scored the highest final germination percentage (FGP) among all intact seeds treatments whereas the control treatment scored the lowest FGP. Soaking in water and H_2SO_4 also significantly ($P < 0.05$) increased the FGP of *C. senegalensis* seeds compared with the control treatment. In treatments using scarified seeds, all treatment except immersion in 4% KNO_3 significantly ($P < 0.05$)

increased the FGP and ranged from 82 to 98.5%. Although, immersion of scarified seeds in 4% KNO_3 significantly ($P< 0.05$) scored higher FGP than the control treatment.

The germination speed (germination rate index (GRI) and corrected germination rate index (CGRI)) and time to reach 50% final germination (GT_{50}) were significantly ($P< 0.05$) affected by all pre-sowing treatments. Mechanical scarification of intact seeds with sand paper significantly ($P< 0.05$) increased GRI and CGRI and decreased GT_{50} compared with other treatments. The control treatment scored the lowest GRI and CGRI among the pre-sowing treatments. Soaking seeds on hot water for 30 min and in H2SO4 for 5 min significantly increased GT_{50} and scored the highest days to reach 50% germination. The results also revealed that increasing exposure time of seeds to H_2SO_4 significantly ($P < 0.05$) decreased GT_{50}. Mechanical scarification of intact seeds and soaking seeds in golden leaf extract treatments scored the lowest GT_{50} (2 days).

DISCUSSION

Seed dormancy is an innate seed property that defines the environmental conditions in which the seed is able to germinate [17]. Otherwise, characteristics of seeds as coat thickness and coat hardness, time and conditions where they are stored and treatment previously offered are involved at sprouting. The present study evaluate some pre-sowing treatments to break dual physical and physiological dormancy of *Crotalaria senegalensis* seeds. The results of the present study revealed that mechanical significantly break the physical dormancy of *C. senegalensis*. This response provide evidence that the seed coat of the plant is the main inhibitor of germination. The mechanical scarification with sand paper significantly increased germination percentage and germination rate and promote germination of dormant seeds of *C. senegalensis*. This results are in harmony with [18]. Although mechanical scarification was an effective treatment for promoting germination but the present study and another study carried by [18] revealed that this method was labor intensive and many seeds were broken in the scarification process due to the small size of Crotalaria seeds. This make the mechanical scarification inefficient for large quantities of seeds. Moreover, [19] found proved the insufficiency of mechanical scarification for other legume such as lupine. The response of H_2SO_4 as a method for breaking seed dormancy in this study was consistent with other studies in different species [20] [21] [22] [23]. Some researchers [24] [25] reported that the seeds of Crotalaria obtained from a natural environment and the H_2SO_4 scarification treatments simulated pass of the seeds through the digestive tract of animals (birds and rodents), which under natural conditions execute chemical scarification. Although the acid scarification significantly broke the dormancy of crotalaria seeds and enhanced germination , but it is commonly not preferred due to its cost, safety risk and environmental precautions involved, and not reliable or lacking the requisite qualities on seeds of other important plant species [26] [27] [28]. In treatments using intact seeds, soaking in hot water increased GP and GRI and decreased GT_{50}. Many researchers [29] [30] found that soaking seeds in hot water for specific period break exogenous seed dormancy due to making scratch in hard seed coat which facilitate the imbibition, moreover this treatment enhance seed germination in many plant species. The present study reveals that crotalaria species has physiological dormancy due to the positive response to chemical treatment KNO_3. The concentration 1% and 2% of the nitrate treatments enhanced germination compared with the highest concentration 4% of the nitrate which might simulated the case in the soil after rainfall which dilutes nitrate and make it available for seed. Supporting evidence was reported by [31] [32] [33]. Nitrate has been stated as being a growth-regulating substance in some plant species such as Salvia [34]. The results of the present study also revealed the importance of golden leaf purslane in breaking dormancy and promoting germination of crotalaria seeds. Some species extracts have high levels of natural plant growth hormones, especially auxins, cytokinins and gibberellins. Gibberellin hormones are very small signal molecules that break seed dormancy and help steer seed germination im many plant species [34] [35].

CONCLUSION

The present study reveals that crotalaria exhibit physical or exogenous dormancy and is entirely imposed by the hard seed coat. The integument is able to withstand unfavorable conditions such as heat, teeth of dispersing agent and mechanical damage prevailing in the natural habitat. This avoidance of germination is ecologically advantageous to the plant grows in harsh climatic conditions , in that seed accumulate in the soil to increase the chance that some will germinate and create new population to maintain the species. But this is limiting when quick and consistent seed germination is desirable for successful establishment of economically important forage plant species. Our results demonstrate that mechanical scarification and soaking in water for 24 h , soaking in golden leaf purslane extract for 24 h or immersion in 1% KNO_3 solution break dormancy and promote germination of Crotalaria senegalensis.

Moreover, our results has established a successful methodology for overcoming seed dormancy and optimizing seed germination of *Crotalaria senegalensis* in order to satisfy the demand for fresh materials as a forage in rangeland and pastures..

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