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Selected Seed Pretreatments on Germination of Kuwait's Native Perennial Plant Species

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Abstract: In this study, the optimal conditions for the seed germination of 3 perennial species were determined based on their responses to mechanical scarification, combination of scarification and GA_3 and dry heat treatment. The desert perennials *Aeluropus lagopoides*, *Teucrium oliverianum* and *Convolvulus oxyphyllus* do not germinate easily and uniformly because of dormancy. A complete randomized designed laboratory experiments was conducted in petridishes at room temperature (22-24°C). Seed germination of *Convolvulus oxyphyllus* and *Aeluropus lagopoides* was promoted by scarification, but not in *Teucrium oliverianum*. In *Teucrium oliverianum*, scarification followed by soaking in 1000 ppm GA_3 enhanced germination from 26% in the control to 43%. Scarification broke dormancy of *A. lagopoides* and *C. oxyphyllus* seeds. The results about the seed germination of these species will help in the mass propagation, restoration and reintroduction programmes.

Key words: Conservation, dormancy, germination, scarification, stratification

INTRODUCTION

Kuwait is a small arid country situated in the North Eastern part of the Arabian Peninsula. Summers are hot, with day time temperatures often reaching 50°C. Winters are relatively cool, with temperatures sometimes falling below freezing, with a mean of between 10 and 20°C. Annual rainfall ranges between 20 and 260 mm and is usually restricted to the period from October to April (Halwagy *et al*., 1982). Perennial plants can often act as effective obstacles to wind blow sand and as the sand particles are checked, a mount of sand becomes established around the base of these plants (Brown, 2003). Water availability is a major factor limiting the regeneration of arid and semi arid species (Adams, 1999).

Convolvulus oxyphyllus Boiss. (Convolvulaceae) locally known as Ethris or Udhris is a shrubby perennial plant grows in coarse sandy soil (Omar *et al*., 2000). The seeds are elliptic, brown or black in color with non prominent hilum. *Teucrium oliverianum* Ging. ex. Benth. (Lamiaceae) locally known as Gasbaa is an aromatic perennial herb grows in stony ground along the edge of Wadi Batin (Omar *et al*., 2000). The seeds are hairy, obliquely bean shaped, one side rounded and the other side has a hollow depression in the centre. *Aeluropus lagopoides* (L.) Trin. Ex. Thwaites. (Poaceae) Locally known as Ikrish. It is a stoloniferous perennial grass with C4 photosynthesis (Bor, 1970; Breckle, 1983; Watson and Dallwitz, 1992). The seeds are smooth, brown and oblong with circular dark hilum. It has attracted interests due to its potential value as an animal forage plant (Torbatinejad *et al*., 2000) and in preventing soil erosion by forming a thick network of roots (Tewari, 1970). The low salt content of *Aeluropus lagopoides* shoots is advantageous in its use as a fodder crop (Gulzar and Khan, 2001). Several uncontrolled factors may influence germination percentage in arid and semiarid natural environments particularly seed dormancy (Mojeremane and Kgati, 2005). The seeds of *Aeluropus lagopoides, Teucrium oliverianum* and *Convolvulus oxyphyllus* are dormant. Dormancy characteristics of a species or a particular population are often assumed to be the result of adaptation to the particular habitat where the species occurs (Schutz, 2000). Understanding the seed germination is crucial for mass propagation, revegetation and restoration, however currently, no information is available on the germination of *Convolvulus oxyphyllus* and *Teucrium oliverianum* species. Little information is available on the effect of salinity and osmotic potential of *Aeluropus lagopoides*. Germination is a complex physiological process controlled by a large number of genes which are affected by several environmental factors such as light, temperature and the duration of storage (Koornneef *et al*., 2002). Successful introduction and propagation of these perennial species will help to control the degradation of the rangelands. This study

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was therefore designed to determine the effects of seed treatments on germination of *Aeluropus lagopoides*, *Convolvulus oxyphyllus* and *Teucrium oliverianum* species.

MATERIALS AND METHODS

Seed collection: Seeds of *Teucrium oliverianum* were handpicked from natural rangeland population in Salmi (N 29 05' 58.9" ; E 046° 41' 21.4"), western border of Kuwait during 2007 and those *Aeluropus lagopoides* and *Convolvulus oxyphyllus* seeds were collected from Newaiseeb (N 28 32' 38.8" ; E 048° 23' 00.1") and Sabah Al Ahmad Natural Reserve (N 29 32' 35.4" ; E 047° 42' 51.6") southern and north east region of Kuwait, during 2006 and 2005, respectively. The seeds were extracted from the fruits by hand and stored at 4°C until use.

Experiment details: The germination studies of the above mentioned species were conducted under "Establishment of seedbank unit for the native plants of Kuwait" project in the Seed laboratory of Kuwait Institute for Scientific Research during 2007. Two germination experiments were carried out in a complete randomized design at laboratory temperature (22 to 24°C).

In the first experiment, the effects of scarification and the combination of scarification and various concentration of GA_3 on seed germination were evaluated. *A. lagopoides*, *T. oliverianum* and *C. oxyphyllus* seeds were scarified by rubbing with sand paper. The scarified seeds of each species were soaked in Gibberellic acid (100, 250, 500, 750 and 1000 ppm, respectively).

In the second experiment, seeds were placed in a preheated oven for 30 and 60 days respectively at 50°C. This temperature was selected because the soil surface reaches this temperature during summer. The germination experiments were conducted at room temperature (22 to 24°C) in 9 cm diameter disposable petridishes lined with Whatmann No. 41 filter paper moistened with distilled water. Four replicates of 25 seeds were used for each treatment, while untreated seeds were used as control for each experiment. Germination was recorded every day and the seeds were considered germinated when the radicle protruded to the length of 2 mm.

Statistical analysis: Data analysis was conducted by using SPSS 12 statistical program. Mean germination percentage and Standard error of mean were calculated. One way ANOVA was used to determine the effect treatments at $p<0.05$ level and Turkey test was performed to evaluate differences among the means.

RESULTS

In *Aeluropus lagopoides* scarification significantly increased the germination (Fig. 1). However, highest germination of 87% was determined in scarified seeds

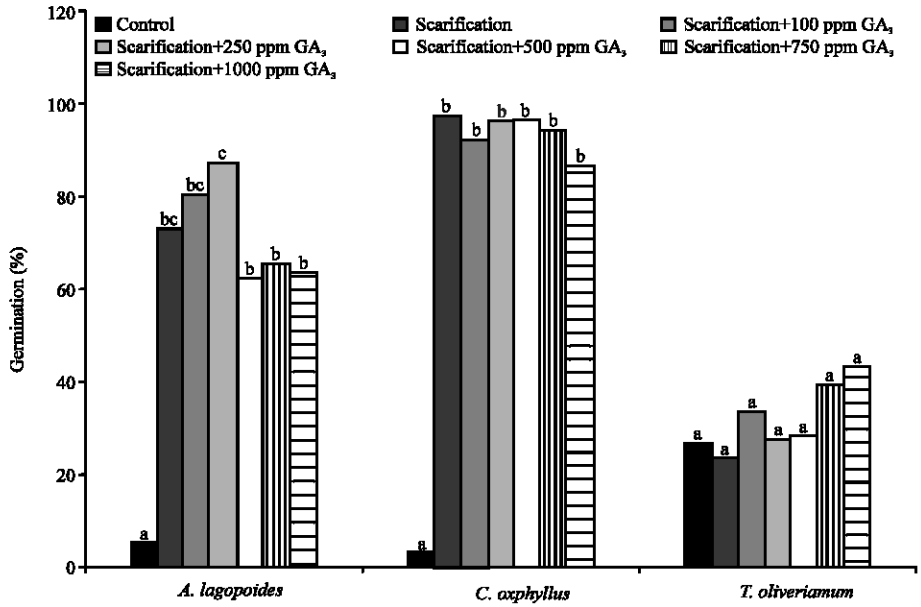

Fig. 1: Effects of Scarification and GA_3 on germination of *Aeluropus lagopoides*, *Convolvulus oxyphyllus* and *Teucrium oliverianum* seeds. Treatment values followed by the same letter are not statistically different at p=0.05

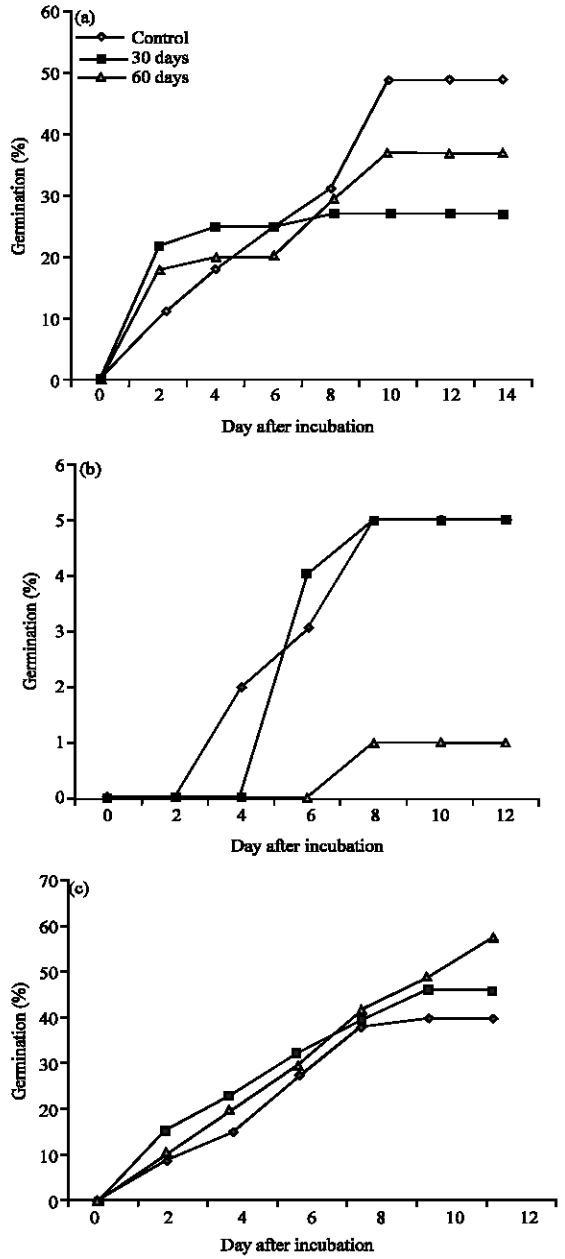

Fig. 2: Germination of (a) *Aeluropus lagopoides*, (b) *Convolvulus oxyphyllus* and (c) *Teucrium oliverianum* seeds in response to heat stratification (50°C) pretreatment

soaked in 250 ppm GA_3 ($p<0.001$). Sand paper scarification significantly increased the germination of *Convolvulus oxyphyllus* seeds from 3% (control) to 97% and it was the most effective pretreatment ($p<0.001$). Furthermore, combination of scarification and GA_3 also resulted in significantly higher germination percentage than that of untreated control (Fig. 1). Increasing GA_3 concentration decreased the germination. In *Teucrium oliverianum* scarification had negative effect on germination (Fig. 1). The germination percentage reduced considerably after scarification ($p = 0.32$). However, soaking seeds in higher concentrations of GA_3 (750 and 1000 ppm) significantly increased ($p<0.1$, $p<0.05$) the germination percentage than the control. In *Aeluropus lagopoides* and *Convolvulus oxyphyllus* seeds increasing GA_3 concentration significantly reduced ($p<0.01$) the germination percentage. In *Aeluropus lagopoides* dry heat treatment reduced germination from 49 to 27% (30 days) and 37% (60 days), respectively (Fig. 2a). In contrast, dry heat treatment increased germination percentage of *Teucrium oliverianum* seeds (Fig. 2c) from 40 to 46 and 58% respectively. The dry heat treatment at 50°C yielded slightly higher germination in *Teucrium oliverianum* seeds, while in *Aeluropus lagopoides* and *Convolvulus oxyphyllus* (Fig. 2a,b) it clearly had a negative effect on germination.

DISCUSSION

Mechanical scarification was the most effective treatment to promote germination and 90-100% of germination was achieved within 24 h in *Convolvulus oxyphyllus*. While in the same time the corresponding percentages for all GA_3 concentrations immersed seeds ranged between 51 and 95%. In previous study, similar result has been reported in *Pterocarpus angolensis* species where the germination was improved by sandpaper scarification than combination with GA_3 (Chisha-Kasumu *et al*., 2007). GA_3 at higher concentration (1000 ppm) highly inhibited germination. GA_3 in high concentrations could be toxic for permeable seeds. Scarification treatment significantly improved germination of *C. oxyphyllus* seeds over that of nonscarified seeds, which coincides with the evidence provided by Sen and Chatterji (1968) for *Convolvulus microphyllus*. In the absence of GA_3, scarification yielded higher germination than controls. Stratification treatments were not effective in improving seed germination, and thus the seeds did not have any physiological dormancy.

In *Aeluropus lagopoides*, scarified seeds plus 250 ppm GA_3 stimulated seed germination (87%). These results are similar with those reported in *Hibbertia commutata* and *Hibbertia amplexicaulis* (Dilleniaceae) in which mechanical scarification increased germination (Allan *et al*., 2004). The germination was further increased by the application of Gibberellic acid solution. Germination percentage decreased as concentration of GA_3 was increased above 250 ppm. The combination of scarification with GA_3 showed a better germination

promoting effect on some taxa of Myoporaceae, Lamiaceae and Myrtaceae than either single treatment (Cochrane *et al*., 2002). It is concluded that *Aeluropus lagopoides* seeds were found dormant and dormancy can be attributed to mechanical and physiological inhibitory mechanism of germination. Dry heat treatment did not have any effect on germination of *Aeluropus lagopoides*. Similar result had been reported in desert perennials *Helianthemum lippii* (Zaman *et al*., 2009) and other *Helianthemum* species (Perez-Garcia and González-Benito, 2006).

A dry heat treatment at 50°C for 30 or 60 days slightly increased the germination of *Teucrium oliverianum* seeds. According to Travlos *et al*. (2007) the dry heat treatment at 50°C improved the germination of *Spartium junceum* seeds. Mechanical scarification inhibited the germination. Similarly in *Cistus creticus*, *Cistus psilosepalus*, *Cistus salviifolius* and *Tuberaria lignosa* scarification was ineffective in promoting germination (Thanos *et al*., 1992). Combination of scarification and high concentration of GA_3 (1000 ppm) slightly improved the germination of *Teucrium oliverianum* seeds. Similar result was observed in *Alliaria petiolata* (Sosnoskie and Cardina, 2009). The germination experiments on *Teucrium oliverianum* revealed that dormancy was not due to mechanical barriers as scarification decreased germination. Where as increasing GA_3 concentration decreased significantly the germination rate of *Convolvulus oxyphyllus* and *Aeluropus lagopoides* seeds, it increased the germination percentage of *Teucrium oliverianum* seeds.

In *Convolvulus oxyphyllus* the highest germination percentages were obtained with manual scarification. Mechanical scarification, resulted in a dramatic increase of germinability and germination is completed in less than a week. On the other hand, heat treatment lowered the germination percentage and it was lower than those of control seeds. In *Aeluropus lagopoides* the combination of scarification and GA_3 (250 ppm) increased germination. Whereas heat treatment (50°C) decreased the germination which was lower than the control. In *Teucrium oliverianum* the scarification decreased the germination percentage which was lower than the control. Although the heat treatment (50°C) and combination of scarification and GA_3 (1000 ppm) slightly increased the germination. These results showed that the effect of dormancy breaking method on germination percentage depends on the plant species.

CONCLUSION

Aeluropus lagopoides seeds exhibit seed coat dormancy (physical) as well as embryo dormancy (physiological). *Convolvulus oxyphyllus* has seed coat dormancy (physical). This physical dormancy favors the accumulation of persistent seed bank in the soil. In *Convolvulus oxyphyllus* and *Aeluropus lagopoides* sand paper scarification is recommended as an inexpensive and effective pretreatment. Scarified seeds of *Teucrium oliverianum* obtained low germination percentage, suggesting that the embryo might have some physiological dormancy. Hence, in *Teucrium oliverianum* further laboratory experiments must be done to enhance the seed germination.

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