SEBHA UNIVERSITY JOURNAL OF PURE & APPLIED SCIENCES VOL.22 NO. 1 2023
DOI: 10.51984/JOPAS.V22I1.2318

مجلة جامعة سبها للعلوم البحتة والتطبيقية
Sebha University Journal of Pure & Applied Sciences

Journal homepage: www.sebhau.edu.ly/journal/index.php/jopas

Methods of breaking seed physical dormancy and germination in native species of *Alhagi graecorum* Boiss (Al-Agool)

Omar Saad Sharashy

Department of Botany, Faculty of Science, Sebha University, Libya

Keywords:
Fabaceae family
Alhagi graecorum
Physical dormancy
Breaking dormancy
Scarification
Germination

ABSTRACT

Alhagi graecorum is a plant belonging to the Fabaceae family and grows as wild perennial shrubby species. Seeds of this species possess seed physical dormancy and need to be removed to enhance germination, breaking of dormancy treatments were imposed on seeds to improve germination. Treatments include scarification with sulfuric acid (H_2SO_4) for 10, 20, 30 and40 minutes. The results showed highly significant difference between control and all the treatments of germination percentage (GP) was 96%, 96%, 97% and 97%, respectively, while recorded in control only 12%. As for the mean daily germination (MDG), the results revealed that, there was a significant differences between all treated seeds and control, which was the fastest and most effective seeds germination on the third day of sowing were 84, 89, 90, 92 % respectively, while in control was 0 %. Mean germination time (MGT) decreased in all treated seed but statistically same while significantly different from the control. The minimum time was recorded for 20 and 30 minutes was 3.14 and 3.14 days respectively.
We conclude from the results obtained that, the use of concentrated sulfuric acid 98% achieved the highest GP and MDG and the lowest MGT. This is the efficient method of breaking seeds dormancy and germination for native species of *Alhagi graecorum.*

طرق كسر السكون الفيزيائي والإنبات لبذور نبات العقول المحلي *Alhagi graecorum* Boiss

عمر سعد شراشي

قسم علم النبات، كلية العلوم، جامعة سبها، ليبيا

الكلمات المفتاحية:
العائلة البقولية
العقول
السكون الفيزيائي
كسر السكون
الخدش
الإنبات

الملخص

نبات العقول (*Alhagi graecorum*) ينتمي إلى العائلة البقولية، شجيري عشبي معمر. تمتلك بذور هذا النوع سكونًا فيزيائيا، ولتعزيز الإنبات بحاجة إلى إزالته، ولكسر السكون لتحسين الإنبات، اشتملت معاملات الخدش باستخدام حمض الكبريتيك المركز لمدة 10، 20 ، 30 و 40 دقيقة. أظهرت النتائج وجود فروق معنوية عالية في كل المعاملات في نسبة الإنبات و التي بلغت 96٪ ، 96٪ ، 97٪ و 97٪ على التوالي، بينما سجلت في الشاهد 12٪ فقط. أما متوسط الإنبات اليومي، فقد أوضحت النتائج وجود فروق ذات دلالة إحصائية بين جميع البذور المعاملة بالحمض مع الشاهد، حيث كان إنبات البذور فيها الأسرع والأكثر فاعلية في اليوم الثالث من البذر فكانت 84 ، 89 ، 90 و 92٪ على التوالي ، بينما في الشاهد 0٪. أما بالنسبة لمتوسط زمن الإنبات، انخفض في جميع البذور المعاملة بالحمض، بينما لم تظهر وجود اختلافات معنوية فيما بينها، وكان الاختلاف معنوياً مع الشاهد. تم تسجيل الحد الأدنى لزمن الإنبات عند المعاملة بحمض الكبريتيك لمدة 20 و 30 دقيقة فقد كانت 3.14 ، 3.14 يومًا على التوالي.

نستنتج من النتائج التي تحصلنا عليها، أن استخدام حامض الكبريتيك المركز% 98 أعطى أعلى نسبة إنبات و أعلى متوسط يومي للإنبات و أدنى متوسط لزمن الإنبات. لذلك تعتبر هي الطريقة الفعالة لكسر سكون البذور و إنبات هذا النوع المحلي لبذور نبات العقول (Alhagi graecorum).

*Corresponding author:
E-mail addresses: osharash@yahoo.co.uk
Article History : Received 30 November 2022 - Received in revised form 22 May 2023 - Accepted 30 May 2023

Introduction

Fabaceae (Leguminosae) is a family with high value of use for food, medicine, forage, ornamental and restoration ecology purposes. One obstacle to the use and management of many legume species is the presence of physical seed dormancy [1] .The genus *Alhagi* belongs to the family Fabaceae (Leguminosae) and is consisted of about nine species [2].The Latin name is derived from the Arabic name *Alhag*, meaning the old wise man [3]. *Alhagi* species are ordinarily used in folk medicine as remedies to treat rheumatism, bronchitis, ulcers, liver disorders and jaundice, urinary tract diseases, asthma and gallbladder problems, recent *in vivo* and *in vitro* biological activity studies on *Alhagi* species revealed their antibacterial, antifungal, antioxidant, antiproliferative, hepatoprotective spasmolytic, and ureter-relaxing effects[4]. *Alhagi graecorum* Boiss, (Camel thorn), known locally as, Al-Agool, is an indigenous plant, shrubby evergreen perennial herb, branched, with rigid spiny twigs, long roots and wide ecological amplitude that allows it to withstand harsh environments, drought tolerant. This species is widely distributed in Murzuq, Ghat, Barkat, Wadi Al Ajal and Al Jaghbub, and grows naturally in xeric successfully and intensively in dry, saline soil, making its in ecosystem desert areas in southwestern Libya, and produces a satisfactory vegetation cover that protects soil from erosion, and it can be highly useful for prevention of land degradation (Fig. 1). It is currently used as a forage for Camels, goats and sheep in a southwest desert regions of Libya (Fig. 2).

Fig. 1: *Alhagi graecorum* Boiss (Habit)

Fig. 2:*Alhagi graecorum*, harvested, collected and sold as livestock forage

Dormancy is a term used to describe a seed that fails to germinate under favorable conditions at a specified time [5], it's can be biologically described as: "The absence of germination of an intact, viable seed under germination favoring conditions with a specific time lapse [6]. Dormancy acts in mimetic-seeded species as an exaptation to reduce seed deterioration. Dormancy is of great importance in an evolutionary perspective and as well in fitness [7],[8], and also important in aspects of dispersal and as a mechanism for delaying seed germination until it has been spread to new areas [9]. Seed dormancy is defined as the failure to germinate under such favorable conditions, although the viable seed [10], [11], and is a temporary failure or block of a viable seed to complete germination under physical conditions that normally favor this [12],[13], it's a block to the completion of germination of an intact viable seed under favorable conditions [14], also it is the resting period of seed after physiological maturity and also an adoption mechanism to overcome stress conditions [15], as well it's important component of plant fitness that causes a delay germination until the arrival of a favorable growth season [16], It's an innate seed property that defines the environmental conditions in which the seed is able to germinate [14],[17]. Seeds of plant species with physical dormancy are known in 17 families of angiosperms [18], and several types of specialized structures ('water gaps') have been found in 12 of the 17 families. Physical dormancy is present in species of at least 15 angiosperm families, including Fabaceae, Malvaceae, Convolvulaceae, Chenopodiaceae, Cannaceae, and Liliciae. In some of these species, seed coat impermeability may delay germination for several years. Physical dormancy is caused mainly by impermeable seed coats that prevent water uptake. The Majority of Leguminosae species have hard and water impermeable seed coats, that inhibits seed germination and causes dormancy. Although hard of the seed coat is a structure which protects the embryo from mechanical effects, it has a negative impact on germination[19].The family of Fabaceae (Leguminosae) has a large number of species with physically dormant seeds [20]. Within the Fabaceae family, the structure associated with the breaking of dormancy is usually the lens[21]. The objective of this experiment was to determine the effective of sulfuric acid (conc.H_2SO_4) on breaking dormancy and promoting germination of *Alhagi graecorum* seeds

Materials and methods

Mature pods of *Alhagi graecorum* were collected from A shrubs growing in different places of the Murzuq region - Libya (N: 55° 25", E: 55° 13", 449 m). (Fig. 3). Seeds were removed from the pods immediately (Fig. 4,5), and stored in glass bottles at room temperature until they were used.

Fig. 3: Map of Libya showing the location of Murzuq

Fig. 4: Mature pods of *Alhagi graecorum*

Fig. 5: Seeds of *Alhagi* ***graecorum***

Chemical scarification

100 Seeds were counted per treatment and soaked separately in sulfuric acid (conc. H_2SO_4 98%) for various time intervals 10, 20, 30, and 40 minutes (min.), to evaluate the time required to break the dormancy of the seeds, scarified seeds were rinsed several times in clean distilled water after the treatment with acids to remove any trace of acid, after rinsing, seeds were allowed to dry on blotter paper at the laboratory temperature, before being placed in Petri dishes. Untreated seeds were used as control. Germination tests, were undertaken with 5

replicates for H_2SO_4, each replicate contained 20 seeds placed in 9 cm sterilized Petri dishes lined with double layered of Whatman No. 1 filter paper. The papers were moistened with 5 mL distilled water and covered-up with their respective covers, and added distilled water was necessary, to prevent seeds from drying out. Afterward, the dishes were incubated in dark at 25 ℃. Seed germination was counted and recorded daily until no further germination occurred. The criterion for germination was visible radicle protrusion from the seed coat [22]. At the end of the incubation time, the following parameters were assessed. Germination percentages were calculated using the following equation.

Germination percentages (GP) $= \frac{G}{N} \times 100$

Where G = Total number of seeds that germinated.

N = Total number of seeds in the Petri dish. [23].

Mean daily germination (MDG), an index of daily germination rate, was determined on the basis of the following equation [24],[25].

$$MDG = \frac{FGP}{D}$$

FGP is the final germination percent. And D is experiment period.

Mean Germination Time (MGT day)

$MGT = \Sigma f \cdot x / \Sigma f$ f = Seeds germinated on day x [26].

Statistical analyses.

Statistical analysis of data and the treatments means were tested by the one way analysis of variance (ANOVA). Mean comparison was performed using Minitab least-significant difference (LSD) method $P < 0.05$ considered significant Values were expressed as means ± SD (standard deviation) for five replicates in each of the independent experiments.

Results and Discussion

From an ecological perspective, dormancy is an important survival mechanism that favors the propagation and dissemination of seeds to establish plant populations. Because specific conditions are required to break dormancy, it may favor germination and seedling emergence under more favorable conditions. In generally treated seeds recorded positive response on germination, the results revealed treatments including scarification with sulfuric acid (H_2SO_4) for different soaking durations were significant effects in all aspects of germination tests. From the results obtained in (Fig. 6) seeds scarified in H_2SO_4 at different durations for 10, 20, 30 and 40 minutes produced high germination percentages (GP) 96%, 96%, 97%, 97% that were highly significant differences to compered with control which showed only 12%. was quite low, while there were no significant differences between the treatments.

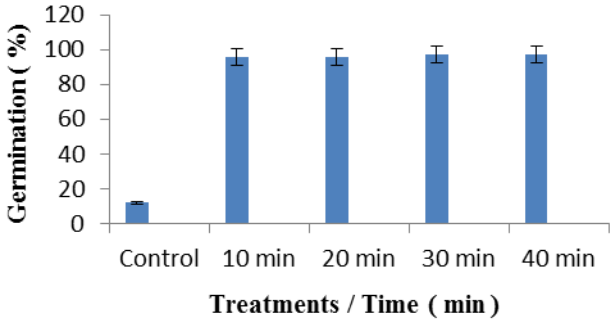

Fig. 6: The effects of different duration of sulfuric acid (98 %) on germination percentage (GP) of *Alhagi graecorum* seeds, Values are means ± (n = 4) ($P < 0.05$)

leguminous family have hard and impermeable seed coats caused by physically dormancy [25]. Generally, legume seeds exhibit hard seededness resulting in dormancy. Several studies have been conducted on legume germination using different seed coat pre-sowing treatments. 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 [27]. Seeds of the fabaceae family exhibit dormancy because of hard testa impermeable to water and gases [15],[17],[28].The results of this study are in agreement with those reported by [29] and [17] showing that, many species of the fabaceae family such as *Lupinus* spp. Seeds exhibit dormancy that is primarily due to water impermeability of the seed's coat. Scarification of Texas bluebonnet (*Lupinus texensis* Hook) seeds with sulfuric acid for 30 to 60 min improved seedling emergence.[27] reported that, 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. The concentrated sulfuric acid treatment has been widely used to improve seed germination of several hard seed coat species [30]. Acids (HCl, HNO3, and H_2SO_4) have been widely used for breaking dormancy of many hard seed coat species, such as European milkvetch (*Astragalus hamosus* L.), blackdisk medick (*Medicago orbicularis* (L.) Bartal. [31] and *Albizia* spp.[30],[17] indicated that, the best treatment to remove hard seed dormancy causing the highest germination percentage was seed scarification with H_2SO_4 and sandpaper. It was observed that for archer and perennial soybeans, using H_2SO_4 immersion was the method that had the highest percentage of germination, so the best method to effect the removal of dormancy [32]. Previous work on *Parkia biglobosa* [33], *Enterolobium contortisiliquum* [34], *Rhynchosia capitata* [17] also showed that soaking of seeds of these plants in H_2SO_4 can break dormancy and increase germination percentage. According to [35], the perennial soybean germination without scarification has a ranging between 7% and 24%, with immersion in H_2SO_4 there was a 100% increase in the germination of their seeds compared to the control which showed only 26% germination. In seeds *Piptadenia-moniliformis* found higher percentages of germination in treatments subjected to immersion in sulfuric acid for 20, 25 and 30 min [36]. According to [37], scarification of seed coat with acids such as H_2SO_4 usually leads to the elimination of exogenous dormancy. These findings are consistent with our findings.

Mean daily germination (MDG), is an index of the daily germination rate. In (Fig.7) the results exhibited that, there were highly significant differences in mean daily germination of germination percentage on the third day of sowing in the treated seeds, 10, 20, 30 and 40 mins compared with control. It was the fastest and most effective in germination achieved 84, 89, 90, 92 % respectively, while in the control was 0 %.

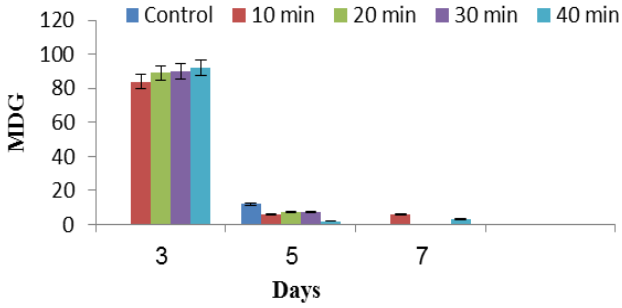

Fig. 7: The effects of different duration of acid (98 %) on mean daily germination (MDG) values of *Alhagi graecorum* seeds ($P < 0.05$)

Mean germination time (MGT) is an accurate measure of the time taken for a lot to germinate, but does not correlate this well with the time spread or uniformity of germination. It focuses instead on the day when most germination events occurred [26] , it is interpreting the time taken to achieve the most germination of seeds.

The results in (Fig. 8) revealed that, the germination time (MGT) was decreased in all treatments of H_2SO_4, 10 min, 20 min, 30 min

and 40 min were statistically the same but significantly different from the control

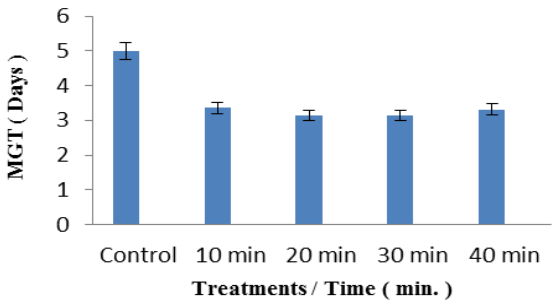

Fig. 8: The effects of different duration of sulfuric acid (98 %) on mean germination time (MGT) values of *Alhagi graecorum* seeds ($p < 0.05$).

The minimum MGT recorded in treated seeds in 20 min and 30 min were 3.14 and 3.14 days respectively. These results are consistent with [38], reported that the lowest MGT values in *Medicago scutellata* and *Medicago polymorpha* species were observed in the seeds treated with H_2SO_4, treatments for 20, 30 min revealed that, H_2SO_4 were adequate to break the hard seed coat of *Alhagi graecorum* seeds in order to induce germination. The results of this experiment confirmed that the *Alhagi graecorum* seeds were in a dormant state. Scarification with H_2SO_4 overcame seed dormancy and increased the germination percentage (Fig.9). It can be stated that the most effective chemical scarification method in breaking seed dormancy of *Alhagi graecorum* is H_2SO_4 treatments. GP, MDG and MGT values at the end of all treatments were found to be quite high, while MGT value was quite low when compared to the control (Fig. 10).

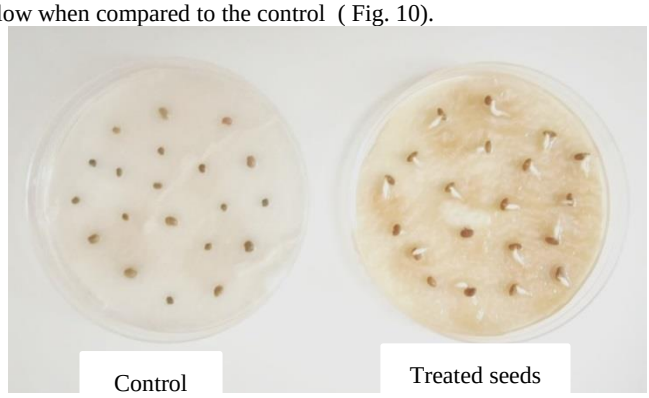

Fig. 9: The comparison in the germination percentage in seeds of *Alhagi graecorum* between control and seeds treated with conc. H_2SO_4 acid 3 days after sowing

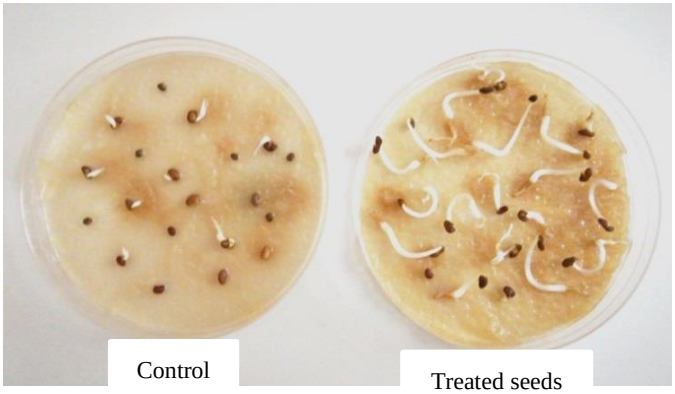

Fig. 10: The comparison in the germination percentage in seeds of *Alhagi graecorum* between control and seeds treated with conc. H_2SO_4 acid 5 days after sowing

Conclusion

The results of the current study demonstrated that seeds of *Alhagi-graecorum* exhibits dormancy imposed by a hard seed coat. Softening of the seed coat by scarifying with concentrated H_2SO_4 significantly increased seed germination percentage (GP), mean daily germination (MDG) and reduced mean germination time (MGT). scarification with H_2SO_4 was the fastest and the most effective dormancy breaking methods for *A. graecorum* seeds.

Therefore, I recommend to use concentrated sulfuric acid when germinating the seeds of the native species of *Alhagi graecorum*.

Acknowledgments

I would like to express to Dr. Ali khair Saber, my sincere thanks and greatest gratitude for his participation in the statistical analysis part of this work.

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