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Impact of Hydrochloric Acid (HCl) on Breaking Seed Dormancy and Germination Enhancement in Native *Alhagi graecorum* Boiss (Al-Agool)

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ABSTRACT

Despite the great importance of legume plants, most of them have a hard and impermeable testa, which causes physical dormancy of the seeds and prevents germination even when environmental conditions are favorable. This is considered one of the major problems in successful seed germination. *Alhagi graecorum* plant is a wild native legume. It has a hard and impermeable testa, which causes physical dormancy of seeds, and to overcome hard coat imposed dormancy, softening the hard seed coat by soaking Hydrochloric acid (HCl). The present study was conducted to evaluate various duration of soaking seeds in HCL to breaking physical dormancy for A.graecorum in 10, 20, and 30 minutes. The results showed that, final germination percentage (FGP) in treated seeds significantly increased when compared with the control were 55, 62 and 68 % respectively, while in control was only 12 %. Seed germination influenced by HCL is due to its capability to break the hard seed coat that leads to water absorption and imbibition of seeds. As for the mean daily germination (MDG), the results revealed that, there were significance increased in seventh day of sowing in germination between the treated seeds in duration of soaking 20 and 30 min were 23 and 24 % respectively, as they were most effective in germination, while the germination in the control stopped in the fifth day of sowing at 12 %. While the mean germination time (MGT), the results indicated that the seeds soaked in acid recorded 9.66, 9.13, and 8.62 days, respectively. The minimum MGT for treated seeds was detected in 30 min., 8.62 days. The reason for the long period of the mean germination time may be due to the use of a low concentration of hydrochloric acid, so I recommend using higher concentrations of acid to obtain a lower mean germination time.

تأثير حمض الهيدروكلوريك على كسر سكون البذور وتحسين الإنبات لنبات العقول المحلي(*Alhagi graecorum*)

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الكلمات المفتاحية:
الفصيلة البقولية
العقول
السكون الفزيائي
غلاف البذرة الصلبة
الخدش
الإنبات

الملخص

على الرغم من الأهمية الكبيرة للنباتات البقولية، إلا إن معظمها لبذورها غلاف صلب ويعتبر عائق في نجاح إنبات البذور العقول (*Alhagi graecorum*) هو من البقوليات المحلية البرية ينمو وينتشر بمنطقة الجنوب الغربي من ليبيا، وللتغلب على ذلك نقعها في حمض الهيدروكلوريك (HCl). أجريت هذه الدراسة لتقييم مدى تأثير الفترة الزمنية للنقع في حمض الهيدروكلوريك عند 10 و20 و30 دقيقة. أظهرت النتائج وجود فروق معنوية في نسبة الإنبات الكلي للبذور المعاملة بالمقارنة مع الشاهد فقد سجلت 55 و62 و68٪ على التوالي بينما كانت فقط في الشاهد 12٪. ويرجع السبب في ذلك على قدرة الحمض على تليين غلاف البذرة الصلب مما سمح بامتصاص الماء وتشرب البذور ونفاذ الهواء. أما بالنسبة لمتوسط الإنبات اليومي، فقد كشفت النتائج أن هناك زيادة معنوية في اليوم السابع من البذر في الإنبات بين البذور المعاملة عند الفترة الزمنية 20 و30 دقيقة كانت 23 و24٪ على التوالي، حيث كانا أكثر فاعلية في الإنبات، بينما توقف الإنبات في الشاهد في اليوم الخامس من البذر بنسبة بلغت 12٪. بينما متوسط زمن الإنبات أشارت النتائج إلى أن البذور المنقوعة في الحمض سجلت 9.66 و9.13 و8.62 يوم على التوالي. والحد الأدنى في متوسط زمن الإنبات وجد في البذور المعاملة عند الزمن

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30 دقيقة كان 8.62 يوم. يعود السبب ربما في طول الفترة في متوسط زمن الإنبات لاستعمال التركيز المنخفض من حمض الهيدروكلوريك، لذا أوصي باستخدام تراكيز أعلى مستقبلاً للحصول على متوسط زمن إنبات أقل.

Introduction

The term dormancy is used to describe a seed that fails to germinate under favorable conditions at a specified time [1]. Seed dormancy is an important component of plant fitness that causes a delay in germination until the arrival of a favorable growing season.[2]. Dormancy of seed is the resting period of seed after physiological maturity and also an adoption mechanism to overcome stress conditions [3]. Many seeds fail to germinate after processing and placement in favorable growing conditions such as seeds are said to be dormant. In general, there are two types of seed dormancy, seed coat dormancy and internal dormancy. Although a hard seed coat is a structure that protects the embryo from mechanical effects, it has a negative impact on germination [4]. Seeds with seed coat dormancy usually have a seed coat that is impermeable to oxygen and water. Physical dormancy is caused mainly by impermeable seed coats that prevent water uptake. Seeds of species with physical dormancy are known in 17 families of angiosperms [5]. Physical dormancy is present in species of at least 15 angiosperm families, including Fabaceae. The majority of Fabaceae / Leguminosae species have hard and water-impermeable seed coats, seed coat dormancy is common in members of the legume family.

The importance of this research is to find out the most appropriate methods to overcome the seed physical dormancy in legume plants, which is one of the most important obstacles that prevent the process of seed germination

This study aimed to evaluate the effects of Hydrochloric acid (HCl 37%), on breaking seed dormancy and enhancing germination for native *Alhagi graecorum*

Materials and Methods

Mature pods containing seeds of *Alhagi graecorum* were collected from plants growing in different places of the Murzuq region in the southwest of Libya, N: 55° 25′, E: 55° 13′, 449 m (**Fig.** 1). Seeds were removed from the pods immediately (**Fig.** 2), and stored in glass bottles at room temperature until they were used.

Chemical scarification. 100 Seeds were counted per treatment and soaked separately in Hydrochloric acid (HCl 36%) for various time intervals of 10, 20 and 30 minutes, to evaluate the time required to break the physical dormancy of the seeds, scarified seeds were rinsed several times in clean distilled water after the treatment 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, each replicate consisted of 20 seeds per Petri dish placed in 9 cm sterilized Petri dishes lined with double-layered 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, when necessary, to prevent seeds from drying out. Afterwards, the dishes were incubated in the 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 [6]. At the end of the incubation time, the following parameters were assessed.

Fig. 1: Map of Libya showing the location of Murzuq region

Fig. 2: (a) *Alhagi graecorum* Boiss (Habit). (b) Pods (c) Seeds

Germination percentages were calculated using the following equation.

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

Where G = Total number of seeds that germinated.

N = Total number of seeds in the Petri dish [7].

Mean daily germination (MDG), an index of daily germination rate, was determined based on the following equation [8, 9].

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

FGP is the final germination percent, and D is the experiment period.

Mean Germination Time (MGT, day)

$$\text{MGT} = \Sigma\, f \cdot \text{x} \,/\, \Sigma\, f$$

f = Seeds germinated on day x [10].

Statistical analyses

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

Results and Discussion

Well-known most legume species exhibit physical dormancy of their seeds and only germinate after exposure to specific environmental conditions. The wild and native seeds of legumes have a hard and impermeable test, which causes physical dormancy and prevents them from germinating even when environmental conditions are favorable [11].

The results of this study in (**Fig.** 3) indicated that the germination percentage was significantly impacted by scarification and all scarification treatments with HCl improved the germination capacity. Seeds sacrificed at different duration, 10 min, 20 min and 30 min, produced high germination percentages of 55, 62 and 68% respectively, which were significantly different compared with the control was 12%. Hydrochloric acid scarification treatments were significant to break seed dormancy and enhanced germination percentage. Legume seeds exhibit dormancy because of hard testa impermeable to water and oxygen. so, seed dormancy of hard seeds can be removed by puncturing the seed coat with acid. This result agrees with [12], who mentioned when seeds were scarified with HCl (36%) seed germination significantly ($p < 0.05$) increased over the control. The results obtained in (**Fig.** 4) showed that the mean germination time (MGT) in the seeds soaked with hydrochloric acid (HCl) at 10, 20 and 30 min. were 9.66, 9.13 and 8.62 days, respectively, while in the control was 5 days, in which it stopped germination on the fifth day of sowing. MGT decreased with increased soaking duration. The minimum MGT detected in seeds treated with 30 min. was 8.62 days.

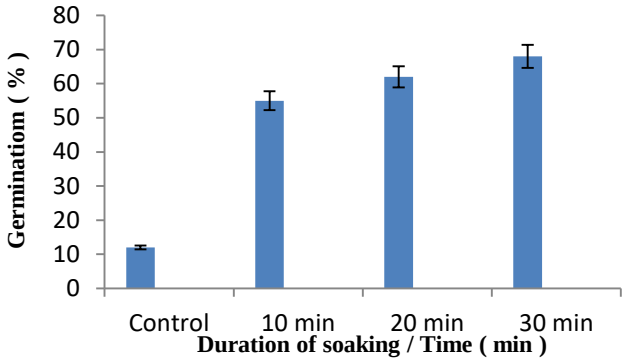

Fig. 3: The effects of different acid treatments (HCl) on germination percentage (GP) of *Alhagi graecorum* seeds ($p < 0.05$)

These results are in line with [13] who found that Bengal dayflower seeds exposed to HCl soaking treatments successfully germinated with little loss of viability after each treatment. The mechanism of possible seed germination influenced by HCl is due to its capability to break the seed coat which leads to water absorption and seed imbibition. The scarification treatments, weaken the cell walls, allowing to water enter the seed hydrated and increasing metabolic activity, particularly enzymes in the endosperm or cotyledons for the synthesis of new materials and embryo growth [14, 15]. The break ofphysical dormancy by treatments of scarification could have an effect on the composition, distribution of nutrients and enzymatic activity during dormancy release [11]. Many scarification treatments have been explored to break physical dormancy in legumes, but the use of acid substances appears to be the most effective method. Indeed, such treatment allows a significant increase in germination in many Fabaceae [16]. This is particularly important for the legume family, where the role of seed coat in imposing physical dormancy has been widely recognized [16-18].
Mean daily germination (MDG), the result revealed that, there was a significantly increased in MDG on the seventh day of sowing in germination between the treated seeds in the duration of soaking, 20 and 30 min were 23 and 24 % respectively, as they were most effective in germination, while the germination in the control stopped in the fifth day of sowing at 12 % (**Fig.** 4).

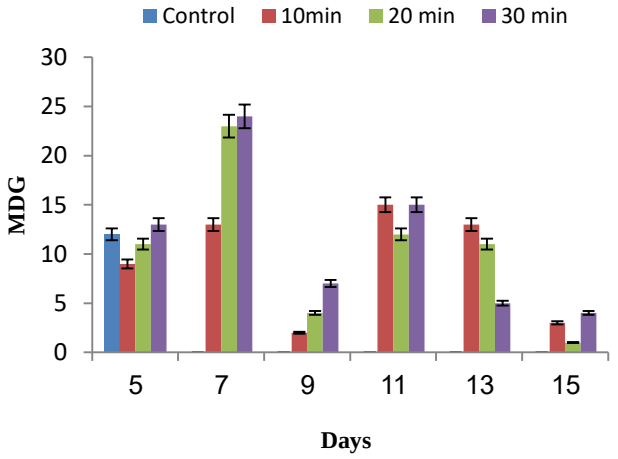

Fig. 4: The effects of different acid treatments (HCl) on mean daily germination (MDG) values of *Alhagi graecorum* seeds ($P < 0.05$)

It was observed the seeds treated with dilute hydrochloric acid had a longer mean germination time than the control. The reason for this may be due to the concentration of the acid. Therefore, I suggest using higher concentrations of hydrochloric acid to break dormancy in the legume seeds, which may give higher germination percentages and a lower mean germination time than the results obtained in this research, based on the use of diluted concentrations of acid.

It can be said that the application of Hydrochloric acid treatments can release physical dormancy in native *Alhagi graecorum* seeds, and the effectivity of these scarification treatments to promote germination range from 12 to 68 %.

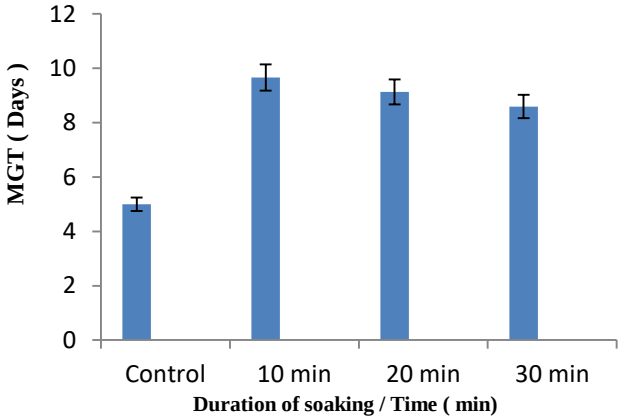

Fig. 5: The effects of different acid treatments (HCl) on mean germination time (MGT) values

Conclusion

It is concluded from the results of the current study *Alhagi- graecorum* seeds exhibit dormancy imposed by a hard seed coat. Softening of the seed coat by scarifying with Hydrochloric acid 36% significantly increased seed germination percentage and mean daily germination. It was observed the seeds had a long period of mean germination time. Therefore, using higher concentrations was recommended to obtain a lower mean germination time.

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