



Comparing dormancy breaking treatments in *Parkinsonia aculeata* seeds

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ABSTRACT

Aims: *Parkinsonia aculeata* is a valuable medicinal plant in traditional medicine adapted to tropical and subtropical arid regions and planted as an ornamental plant. Since seed germination of *P. aculeata* does not occur quickly, the current research was performed to test different dormancy-breaking treatments on germination characteristics of *P. aculeata*.

Materials and methods: The studied treatments included scarification with sandpaper, H₂SO₄ (98%), KNO₃ (0.2%), soaking the seeds in 90 °C hot water for 15 minutes, Gibberellic acid (250, 500, and 100 ppm), leaching (placing the seeds in running water for 48 hours), a combination of leaching treatment with KNO₃, and combination of leaching treatment with Gibberellic acid (250, 500 and 100 ppm). To compare the results, the distilled water was considered as a control treatment. A Completely Randomized Design (CRD) was made with 13 treatments and 4 replications.

Results: Results showed significant differences between treatments on germination percent, germination speed, length of root, length of shoot and length of seedling, and index of seed vigor (p<0.01). The seeds had more than 85% dormancy, and applying to leach (germination percent, 75%) and scarification treatments (70%) as well as boiling water (57.5%) had the highest effect on releasing the seed dormancy compared to control (15%).

Conclusion: Since this plant's establishment problems are the seed dormancy period, using leaching for 48 hours will help in the germination improvement of *P. aculeata*.

Keywords: Germination; Gibberellic acid; Leaching; Scarification; Seed dormancy.

CITATION LINKS

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Introduction

One of the essential medicinal species that has grown in Iran is *Parkinsonia aculeata*. This species belonged to the Leguminosae family and subfamily of Caesalpinaceae. *P. aculeata* is native to the tropical region of America and is also found in India, Pakistan, and southern Iran. This plant has been mentioned as an astringent, healing, and analgesic in traditional Iranian medicine, and its bark is used in the paper industry. In some southern regions of Iran, the leaves and shoots of the plant are used as a poultice to neutralize the effects of various scorpion venom [1]. Due to the harmful side effects of chemical drugs, using drugs of plant origin has increased. This increasing tendency causes the demand for enhancing the area under cultivation of these plants to achieve more production than before. Therefore, it is necessary to remove barriers to the cultivation and production of medicinal plants [2]. Seeds of wild plants, including medicinal plants, usually have more intense dormancy than domestic species [3, 4].

Seed dormancy is a temporary strategy in seed life that enables it to complete its germination under favorable conditions. Different types of seed dormancy, including physiological, physical, or morphological dormancy, are due to reasons such as the layers covering the embryo, undifferentiated embryo, or immature, and ultimately due to metabolic limitations [4]. It is known that legume seeds have physical dormancy [5], and many researchers have used different methods to release seed dormancy [6-13]. Rusdy [8] reviewed papers about the dormancy breaking of tropical forage legumes and found that the type of seed dormancy mainly was physical dormancy which acid scarification was the most efficient treatment to break seed dormancy, followed by sandpapering and hot water. Another study on determining the kind of seed dormancy in the legume

genus *Cassia*, Rodrigues-Junior et al. [10] reported physical dormancy as the only kind of seed dormancy found for 53 *Cassia* species. Researchers studying the breaking seed coat dormancy of *Ulex europaeus* seeds concluded that treating the seeds with sulfuric acid and sandpaper increases seed germination [14]. Another research has shown a positive effect of scarifying on breaking dormancy and stimulation of germination of some alfalfa seeds [15]. A similar experiment on *Acacia farnesiana* seeds showed that the application of sulfuric acid increased the germination of the seeds, but increasing the duration of seed contact with acid increased abnormal seedlings due to damage to the structure of the seed embryo [16]. In addition to mentioned legume species, *P. aculeata* also has physical seed dormancy (>80% fresh seeds when inundated at 20 °C), and studies were tried to release the dormancy [17-22]. Cochard and Jackes [23] reported that dormancy release *P. aculeata* increased with increasing temperature in all populations. The averaged responses were significantly different between Costa Rican and Australian seed populations and between seeds collected from the soil and trees; the germination rate of scarified seeds was fastest at 35 °C in all seed populations. Van Klinken et al. [22] conducted a seed burial trial to investigate dormancy release of *P. aculeata* across the entire environmental distribution in Australia (arid to wet-dry tropics, uplands to wetlands, soil surface to 10 cm deep). Their results indicated that dormancy release was quickest for seeds buried during the wet season at relatively high rainfall, upland sites (only 3 % of seeds remained dormant after 35 d). The longest-lived seeds were in wetlands (9 % remained dormant after almost 4 years) and on the soil surface (57 % after 2 years). A wide range of mechanisms was used to release dormancy of *P. aculeata* seeds, including intense dry

heat [24], physical damage to the seed coat, and artificial methods such as boiling water and acid scarification [17-19, 23], wet heat (exposure to wet, warm to hot conditions under field conditions) [22].

Although *P. aculeata* introduced as invasive species [23, 25], it has many benefits in arid zones with a harsh climate, and it is used as an ornamental tree in towns and botanic gardens and also as a hedging plant and for shade, windbreak, soil nitrogen fixation and rehabilitation purposes [25] and also for medicinal value [1]. As mentioned, the first limitation for the cultivation of plants with seed dormancy spatially in arid regions is to achieve the best method for breaking seed dormancy to improve germination and mass production. Therefore, this study aimed to compare different methods of breaking seed dormancy in *P. aculeata* seeds in Zabol city, Sistan region.

Materials and methods

In order to evaluate the effect of different treatments on breaking seed dormancy and seed germination of *P. aculeata*, an experiment was conducted at Zabol University in 2018. First, seeds were collected from the growing areas of the species in Sistan (Suburb of Zabol city) in the summer of 2018. Preliminary tests showed that *P. aculeata* seeds had initial dormancy and could not germinate under normal conditions. Therefore, according to Mohnot and Chatterji [17], Teketay [19], van Klinken [20], and Cochard and Jackes [23], different treatments were used to eliminate seed dormancy. Treatments include control (distilled water), scarifying the seed coat with sandpaper, treating the seeds with 98% sulfuric acid for 10 minutes, soaking the seeds in 0.3% potassium nitrate for 48 hours, soaking the seeds in 90 °C hot water for 15 minutes, soaking the seeds in gibberellic acid at 250, 500 and 1000 ppm

for 12 hours, placing the seeds in running water for 48 hours (leaching), combining leaching treatment for 48 hours and 0.3% potassium nitrate, the combination of leaching treatment for 48 hours and 98% sulfuric acid, the combination of leaching treatment for 48 hours and gibberellic acid with 250, 500 and 1000 ppm.

Before starting the experiment, the seeds were disinfected with 10% sodium hypochlorite solution and then washed several times with distilled water. At the end of the soaking period, all seeds were washed with distilled water, and after drying, they were placed in 9 cm Petri dishes on Whatman's filter paper No. 1 for germination. Before placing the seeds, the Petri dishes were first sterilized in the oven for 48 hours at 20 ° C. Germination test was performed in an experiment in a completely randomized design with 4 replications (10 seeds per replication) in the germinator at a temperature of 25 ° C. During 15 days, germinated seeds with a root length of higher than 2 mm were counted every day, and germination percentage, germination speed, radicle, plumule, and seedling lengths, and seed vigor index were measured. Germination percentage and germination speed [26] were calculated based on the following equations.

$$GP=100\times (G/N) \quad (1)$$

GP: germination percentage, G: number of germinated seeds, N: total number of seeds

$$GR= \sum ni/ti \quad (2)$$

GR: Germination rate, ni: germinated seeds at time ti, ti: number of days after germination

$$\text{Seedling Length} = \text{Root Length} + \text{Shoot Length} \quad (3)$$

$$\text{Vigor Index} = \text{Mean of Seedling length} \quad (4)$$

(mm) × Germination percentage

Experimental data were analyzed using SPSS software. After normalizing the data, ANOVA was performed, and the mean data were compared using Duncan's multiple range tests.

Results

The analysis of variance showed that the effect of different treatments on germination percentage and germination speed, root, shoot, and seedling length, and seed vigor index of *P. aculeata* was statistically significant at a probability level of 1% (Table 1).

Mean comparisons of different treatments on germination traits of *P. aculeata*

The mean comparisons showed that all treatments used in this experiment except sulfuric acid had a higher germination percentage and germination speed compared to the control treatment. The highest germination percentage and speed were obtained by applying leaching treatments for 48 hours (75%) and scraping the seed coat with sandpaper (2.6 seeds/day), respectively. Germination percentage in the control treatment was 15 (percent), and germination speed was 0.4 (seed/day), which was significantly different from other treatments (Table 2). The maximum length of root, shoot, and seedling was also related to leaching treatments for 48 hours. Root, shoot, and seedling length showed an in-

crease of 1.5, 3.5, and 5 cm compared to the control treatment, respectively. Treatments of hot water, gibberellic acid 250 ppm, and scarifying also improved the initial growth of seedlings, which was significantly different from the control treatment. Finally, the shortest length of root, shoot, and seedlings was obtained due to sulfuric acid (Table 2). There is also a significant difference in seed vigor in the treatments of leaching, scarification, hot water, and gibberellins. The highest seed vigor index was obtained in leaching treatment (748), while the lowest was related to sulfuric acid (4.7). The seed vigor index of the control treatment was 75 (Table 2).

Discussion

This experiment shows that the used treatments have a significant effect on increasing the germination percentage and germination speed, which is consistent with the results of van Klinken et al. [22] on the studied species and Asaadi et al. [27] on the other hard seed species. This type of dormancy is mainly related to the physical properties of the seed coat [22-23] and is controlled by external factors. In some cases, the degree of hardness of the seed coat can be due to lipids, tannins, and pectic substances present in the seed [28].

The highest germination percentage and seedling growth were obtained in leaching treatment for 48 hours. Leaching of *P. aculeata* seeds reduced mucilage formation

Table 1) Variance analysis of studied traits of *H. sabdariffa* species

Properties	Germination percentage	Germination speed	Root length	Shoot length	Seedling length	Seed vigor
ss	18180	25	38	204	403	2222938
df	12	12	12	12	12	12
ms	1515	2	3/2	17	33/6	185244
f	20**	89.3**	268.4**	215.5**	444.5**	72.1**

** : significant differences between treatments at 1% level

Table 2) comparing the effects of various treatments on germination traits of *P. aculeata*

Treatment	Germination percentage (%)	Germination speed (per day)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seed vigor
Gibberellic acid (250 ppm)	45.00±2.9bcd	1.55±0.06c	2.5±0.02b	4.6±0.15c	7.05±0.13b	316.4±16.3cd
Gibberellic acid (500 ppm)	32.5±4.8de	0.78±0.08f	1.0±0.04f	1.2±0.04g	2.2±0.08h	70.8±3.2g
Gibberellic acid (1000 ppm)	37.5±7.5cde	1.87±0.12b	1.5±0.07d	4.1±0.2d	5.6±0.14e	213.0±49.0e
KNO ₃	32.5±2.5de	1.3±0.09d	0.5±0.05g	1.5±0.16g	2.0±0.14h	64.2±3.47fg
Scarification	70±4.0a	2.6±0.07a	1.0±0.02f	5.1±0.3b	6.0±0.29d	433.7±45.6b
Leaching	75±2.9a	2.4±0.11a	3.0±0.04a	7.0±0.09a	10.0±0.1a	748.7±34.6a
Boiling water	57.5±2.5b	1.8±0.08b	2.0±0.1c	4.7±0.06c	6.6±0.16c	381.5±12.6bc
H ₂ SO ₄	12.5±2.5f	0.2±0.04h	0.12±0.02h	0.3±0.02h	0.4±0.04i	4.7±0.5g
Leaching + Gibberellic acid (250 ppm)	25.00±5.0ef	0.7±0.2f	0.97±0.02f	3.0±0.2f	4.0±0.16g	99.5±20.4f
Leaching + Gibberellic acid (500 ppm)	55.0±6.5b	1.3±0.08d	1.3±0.08	4.0±0.14d	5.27±0.09ef	290.0±34.5d
Leaching + Gibberellic acid (1000 ppm)	47.5±4.8bc	1.7±0.04bc	1.05±0.06f	3.25±0.03ef	4.3±0.08g	203.7±19.4e
Leaching +H ₂ SO ₄	30.0±4.1e	1.05±0.06e	0.12±0.02h	0.5±0.4h	0.6±0.07i	17.2±1.1g
Control	15.0±2.8f	0.45±0.03g	1.5±0.04d	3.5±0.02e	5.0±0.10f	75.0±15.0fg

The different letters indicate a statistically significant difference ($p < 0.01$).

around the seeds and increased seed germination compared to the control. Therefore, it seems that the factor involved in seed dormancy is inhibitory compounds (mucilage) in the seed coat. Chemical materials accumulated in the fruit and seed coat during seed development stages can remain in these areas even after harvest. These materials act as inhibitors in the germination phenomenon. These inhibitory compounds include phenols, coumarin, and abscisic acid, which can be washed and eliminated by soaking in water [29]. One of the factors affecting the seed

dormancy of plant species is seed coat. In the current investigation, the successful germination of *P. aculeata* seeds under treatments of breaking dormancy confirms the effect of mechanical resistance of the seed coat against seedling emergence, which is consistent with the results of other researchers on the species [20, 22-23]. Different concentrations of gibberellic acid and 0.3% potassium nitrate increased germination percentage and germination speed compared to the control treatment. One of the reasons for the positive effect of chemical stimulants such as

gibberellin and potassium nitrate on seed germination of *P. aculeata* is probably due to the balanced hormonal ratio in the seed and the reduction of growth inhibitors as abscisic acid (ABA) [30].

Gibberellic acid as a chemical stimulant can disrupt the physiological dormancy of seeds. Gibberellic acid initiates germination by inducing the synthesis of the alpha-amylase enzyme, resulting in the breaking of seed dormancy. Potassium nitrate removes dormant seeds needing light and is an effective chemical in reducing light requirements and increasing germination. Potassium nitrate helps respond to the metabolic processes of seeds. This compound may induce auxin biosynthesis and initiate the growth of the embryo [31]. Our experiment, scarifying with sandpaper, improved germination percentage and seedling growth in *P. aculeata* compared to the control. Eisvand et al. [32], working on *Astragalus siliquosus* seeds, found about 95% of dormancy is due to hard seed coat so that it is impermeable to water, and sandpaper is the best method to remove it.

Field studies indicated that wet conditions [23] and wet heat [22] had a prominent effect on releasing seed dormancy of *P. aculeata*. This study indicated that the seeds of *P. aculeata* had higher than 85% dormancy, and the treated seeds had the highest germination percentage under the effective treatments on the seed coat, including leaching, scarifying, and hot water.

Conclusion

It can be concluded that the seed dormancy of the studied species is most likely related to the physical factors of the seed coat. According to field studies [22-23] and the results of this study, seed dormancy could be quickly released by leaching for 48 hours. Leaching can be more critical in terms of being cheap with low risk and the

possibility of harming the embryo compared to chemicals, especially acid. Since one of the problems of establishing this plant is the length of seed dormancy, the results of this study will help the germination of this medicinal plant.

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