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Effect of Some Physical Treatments to Overcome Zarin-giyah (*Dracocephalum kotschyi* Boiss.) Seed Dormancy

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ABATRACT

Zarin-giyah (*Dracocephalum kotschyi* Bioss.) is a medicinal plant and an endemic species in Iran. It is grown and well adapted to high elevations. This plant can be found in some areas such as Tabriz, Yasuj, Mazandaran and Isfahan. Germination in Zarin-giyah seed is irregular and takes a long period of time. This study conducted in 2021-2022, and the potential of Zarin-giyah seed dormancy release was evaluated using eight different treatments which consisted of: scratching with sandpaper, 24 hours of running water + 12 weeks of cooling at 4˚C, 48 hours of running water + 12 weeks of cooling at 4˚C, 60 ˚C hot water for ten minutes, freezing the seeds for 48 hours at -18 ˚C, 24 hours of running water + 48 hours of freezing at -18 ˚C, 24 hours of running water + scratching and the control. The result showed that seedling germination index, root and shoot dry weight, shoot length, mean germination time and germination rate were significant at the 1% probability level. Also, these results showed that using 24 hours of running water and 12 weeks of cooling at 4˚C improved germination traits in Zarin-giyah. Reducing the duration of seed immersion in running water is suggested as a means of achieving better results.

***Keywords*:** Germination rate, Medicinal plant, Dormancy-release treatments, Zarin-giyah

INTRODUCTION

Most of the time, medicinal plant seeds are dormant in their natural state. Understanding the seed dormancy influence factors and creating ideal conditions are crucial for the medicinal plants' widespread cultivation (Hatami *et al.,* 2019). *Dracocephalum* is from the Lamiaceae family with more than 60 species. Zarin-giyah is one of the species and an endemic plant in Iran with medicinal properties. It grows in the regions of Isfahan, Chaharmahal and Bakhtiari, Mazandaran, Gorgan, Hamedan, Kermanshah, Lorestan, Tehran, Fars and the northern heights of Semnan (Foroozandeh and Asadi-Gharneh, 2021). Zarin-giyah grows in high rock areas at an altitude of 2300 to 3000 meters above the sea. Topographic and meteorological data have shown that this plant requires highlands and rocks with moderate temperatures in summer, high relative humidity and rainfall between 400-600 mm (Asaadi and Khoshnood-Yazdi, 2010). Their microclimatic conditions and special needs such as rocks with high rainfall and highlands have led to the reparation in small and separate populations. Providing a natural-like environment could facilitate the adaptation of Zarin-giyah to cultivation. Zarin-giyah can be used in plant breeding processes by collecting various ecotypes.

Zarin-giyah is propagated by seeds. Researches shows *Dracocephalum* genus seeds, despite being mature and healthy, may not germinate because of their dormancy (Chang *et al.,* 2009; Hatami *et al.,* 2019). Although *Dracocephalum* is distributed in the central and western regions of Iran, it is classified as an endangered species due to hard seed coats, irregular germination, and seed dormancy issues (Jalali and Jamzad, 1999). Seed dormancy is a more prevalent issue in wild species than in cultivated ones, and this poses a significant obstacle to the widespread cultivation of medicinal herbs (Kochaki and Sirmainea, 2000).

Generally; some factors such as embryo immaturity and unbalanced ratio of the required hormones in plants are cause of seed dormancy (Sarmadnia, 1996). Various researches have shown that using gibberellin could have a good effect on Zarin-giyah seed germination by changing the environmental pH. One of appropriate methods to break seed dormancy is mechanical scraping and using gibberellin. They have shown a significant increase in *Dracocephalum* seed germination (Hatami *et al*., 2019). Some studies show, the use of gibberellin has no significant effect on germination rate alone, which is probably due to the hard coat of the seeds and the high accumulation of mucilage that prevents the hormone absorption by the seeds (Hatami *et al.*, 2019). Ghani *et al.* (2015) reported, the gibberellin with 2000 ppm concentration resulted in the highest percentage of total germination (75%) on Zarin-giyah seeds. According to the morphophysiological seed conditions, some physical treatments can be useful to promote seed germination as an alternative hormonal method (Ellis *et al.,* 1985; Huxley, 1992).

The current study aims to assess various non-hormonal treatments for Zarin-giyah germination and identify the most effective one. This approach was taken in an effort to reduce reliance on hormonal treatments.

MATERIALS AND METHODS

Experiment site

The present experiment was conducted in the laboratory of the Horticulture Department at Isfahan (Khorasgan) Branch, Islamic Azad University during 2021-2022 with a completely randomized design and three replications.

Seed preparation

Zarin-giyah seeds were obtained from the Department of Natural Resources of Isfahan province.

Experimental procedure

In order to carry out the experiment, first the seeds were disinfected with 5% sodium hypochlorite solution for 3 minutes. After washing several times, seed dormancy release treatments were applied (Table 1).

Each petri dish was considered as a treatment. The petri dishes contained 25 healthy seeds that were kept inside the germinator at 25˚C temperature and a photoperiod of 16 hours of light and 8 hours of darkness. The experiment duration was 30 days and during this period germinated seeds were counted every 24 hours. After the end of the experiment, germination properties such as seed germination index (GI), root and shoot dry weight (RDW) and (SDW) respectively, root and shoot length (RL) and (SL) respectively, mean germination time (MGT), germination rate (GR) and germination percentage (GP) were measured.

The seedling of each treatment was placed inside the envelope separately to determine the root and shoot dry weight. Then the samples were put in laboratory oven with 70˚C temperature for 48 hours. The seedling dry weight was measured using a sensitive scale with an accuracy of 0. 001. The length of the root and shoot were measured from the rosette to both ends of the seedling by a millimeter ruler on a flat surface in petri dishes, the average number of them was reported. Other indexes were measured by (Table 2) methods.

Statistical analysis

The statistical calculations of this experiment were done with SPSS software version 22 and Duncan's multiple range test was used to compare the means.

RESULTS

The results show that GI, RDW, RSW, SL, MGT and GR were significant at the 1% probability level (Table 3). Regarding Table 4. the GI results ranged from 8.11 to 1.18 that the best amount was observed in T_3, higher than the control and other treatments. Also; RDW results ranged between 0.041 to 0.008 mg, and T_3 was the highest, however, there was no significant difference between control and other treatments (Table 4).

In SDW the T_6, T_5, T_7 with 0.073, 0.063, 0.061 (mg) respectively were the best results (Table 4). The highest RL were in T_3 and T_2 with 6.66 cm and 6.49 cm respectively and the lowest was in T_6 with 3.90 cm (Table 4). The MGT results ranged from 12.33 to 6.60 and the highest result was shown in T_6 (Table 4). In GR the best result reported in T_8 with 0.83 day^{-1}, T_1 with 0.049 day^{-1} was the lowest amount between treatments (Table 4).

GP percentage was not significant in all treatments (Table 3) and the range was different between 98% to 59.66%. The highest and lowest amount was shown in T_8 and T_1 respectively (Table 4).

DISCUSSION

Germination is an important stage of a plant's life cycle, and the plant path chooses has an effect on its survival. Seed dormancy is a physiological challenge that many medicinal plants face, so removing the seed dormancy is necessary for germination (Jankju-Borzelabad and Tavakkoli, 2008).

The best treatment in this study was T_3 (24 hours of running water + 12 weeks of cooling at 4˚C) that increased indicators such as seed germination index, root dry weigh and root and shoot length. Ghani et al (2015) reported that seed washing beside three weeks stratification can be one of the cheapest methods to improve the quality of seed germination. Other research shows breaking seed dormancy treatments, such as stratification and chilling, result in a phytohormones ratio balancing that has an effect on germination. This is due to the decomposing enzymes release so could activate embryo nutrition after storage and ultimately leading to seed germination (Bewley, and Black, 1994). T4 (48 h running water + 12 wk chilling at 4°C) did not produce acceptable results compare to other treatments, perhaps mucilage production by the seeds affected on oxygen availability, so reduced the seed germination. (Ghani *et al.*, 2015). T_8 (24 hours of running water + scratching) showed the highest germination percentage.

Due to, scratching the Zarin-giyah seed with sandpaper increased the percentage of seed germination compared to the control and other treatments by removing physical factors in the hard shell of the seed. Also, when the seed coat is thinned or removed, water absorption occurs more quickly within a 24-hour period. This leads to the seed germination metabolic activity start and a reduction in the time required for root and shoot emergence, resulting in a speed of the germination process. (De and Kar, 1994).

The high growth index shows the high seedling germination index. Most of the growth properties have increased in T_3, and as a result, the index of seedling growth has also increased (Table 4).

Some studies have shown that chilling and water priming have a positive impact on the germination characteristics of *Alkana tinctoria*, *A. pseudocotula*, *A. haussknechtii*, and *Bellis* perennis. Therefore, these treatments can enhance the germination properties of these particular genus and species. (Sajadi jagharogh, 2012; Sedaghat hoor, 2016).

Although the seed germination characteristics of Zarin-giyah increased in all treatments compared to the control, the improvement was more obvious in T_3. However, the percentage of germination in this treatment did not increase significantly, which could be due to keeping the seeds in running water for a long time. Therefore, reducing the mentioned time can improve the percentage of germination in Zarin-giyah and it can be used as a suitable and favorable offer.

Table 1. Seed dormancy treatments used in this experiment.

T_8	T_7	T_6	T_5	T_4	T_3	T_2	T_1
24 hours of running water + scratching	24 hours of running water + 48 hours of freezing at -18˚C	Freezing the seeds for 48 hours at -18 ˚C	60°C hot water for ten minutes	48 hours of running water + 12 weeks of cooling at 4˚C	24 hours of running water + 12 weeks of cooling at 4˚C	Scratching with sandpaper	Control

Table 2. Indexes and methods

Method	Description	Source
GP= N_i/N ×100	GP: germination percentage, N_i: total number of germinated seeds, N: total number of cultivated seeds	Sedaghat hoor, 2016
GR= ΣN/D	GR: germination rate, N: the number of germinated seeds per day, D: the number of days pass	Agrawal, 1991
GI= PG × W_d	GI: germination index, $W_{d:}$ seedling dry weight	Pasandideh et al, 2014
MGT= ΣDN/Σn	MGT: mean germination time, DN: the number of days counted from the start of the experiment, n: number of germinated seeds	Alvarez and Grigera. 2005

Table 3. Results of analysis of variance for measured traits

S.V	df	Mean square (MS)							
		GI	RDW (mg)	SDW (mg)	RL (cm)	SL (cm)	MGT	GR (day^{-1})	GP (%)
Treatments	7	6.965^{**}	0.000^{**}	0.001^{**}	3.112^{*}	1.059^{**}	11.428^{**}	0.036^{**}	488.857^{ns}
Error	16	0.001	0.000	0.000	0.083	0.000	0.000	0.000	0.250

** and * Significant at 1 and 5% probability level respectively. GP: germination percentage, GR: germination rate, GI: seedling germination index, MGT: mean germination time, RDW: root dry weight, SDW: shoot dry weight, RL: root length, SL: shoot length

Table 4. Comparison of the average of different treatments on *D.kotschyi* germination characteristics

	GI	RDW (mg)	SDW (mg)	RL (cm)	SL (cm)	MGT	GR (Day^{-1})	GP (%)
T_1	1.19^{h}	0.007^{b}	0.013^{e}	5.66^{b}	0.59^{h}	8.8^{f}	0.049^{g}	59.66^{f}
T_2	3.84^{e}	0.006^{b}	0.036^{d}	6.49^{a}	0.96^{g}	6.60^{h}	0.75^{b}	91.66^{b}
T_3	8.11^{a}	0.041^{a}	0.056^{b}	6.66^{a}	2.29^{a}	10.1^{d}	0.69^{c}	83.66^{c}
T_4	3.04^{g}	0.005^{b}	0.046^{c}	4.60^{c}	1.50^{d}	10.47^{c}	0.055^{f}	67.66^{e}
T_5	5.08^{c}	0.008^{b}	0.063^{ab}	4.50^{c}	1^{f}	7.81^{g}	0.60^{e}	71.66^{d}
T_6	6.21^{b}	0.005^{b}	0.073^{a}	3.90^{d}	2.20^{b}	12.33^{a}	0.66^{d}	79.66^{c}
T_7	4.72^{d}	0.003^{b}	0.061^{ab}	5.80^{b}	1.60^{c}	11.80^{b}	0.60^{e}	72^{d}
T_8	3.62^{gf}	0.007^{b}	0.03^{b}	4.60^{c}	1.40^{e}	8.88^{e}	0.83^{a}	98^{a}

GP: germination percentage, GR: germination rate, GI: seedling germination index, MGT: mean germination time, RDW: root dry weight, SDW: shoot dry weight, RL: root length, SL: shoot length.

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