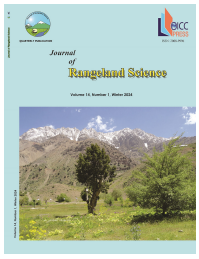

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Efficient Methods in Breaking Seed Dormancy of *Bunium luristanicum*

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Abstract:

Nowadays, in many rangelands, due to overgrazing and climate change, the plant germination and establishment are greatly reduced. This study was carried out to investigate the effects of seed dormancy breaking treatments on *Bunium luristanicum* germination (in winter 2021). Seeds of *B. luristanicum* were subjected to 16 dormancy breaking treatments using a completely randomized design (CRD) with five replications. Result showed that gibberellic acid (GA_3) + hot water (70 °C) and GA_3 + H_2SO_4 (50 %) with average values of 80 % and 82 % had the highest germination percent, respectively. Seed scarification with hot water was better than sulfuric acid because acid increased abnormal seedling number. The highest seedling weight with values of 16.9 and 14.9 mg were recorded in the treatments of GA_3 + sulfuric acid 50 % and GA_3 + hot water 70 °C, respectively. The highest abnormal seedling was obtained in combination of sulfuric acid 75 % with other treatments. The highest seed vigor index was obtained in seeds treated with GA_3 (500 ppm) + hot water 70 °C. Results indicated seeds scarification with hot water 70 °C (10 min) followed by soaking in GA_3 (500 ppm) for 12 hours was the best treatment for seed dormancy breaking, and improved seedling growth of *B. luristanicum*.

Keywords: Gibberellin; Seed germination; Sulfuric acid; Hot water

1. Introduction

Bunium luristanicum is one of the most important species of the family of Umbelliferae. The umbelliferae contains about 3000 species of plants dispersed throughout the world, especially in the North hemisphere (Baskin and Baskin, 2014). Seed dormancy is a physiological phenomenon in wild and cultivated plants, and is more common in wild plants than the cultivated plants (Farahani et al., 2011). Dormancy can keep some plant species in particular environmental conditions, and as one of the most important survival mechanisms in plants, it delays seed germination until conditions of the location and time are suitable for germination. Thus, delay in seed germination is not haphazard, and in many seeds in dormancy, some morphological and physiological changes should occur for germination (Jaganathan, 2020). Thus, in the absence of physiological dormancy, overcoming dormancy may lead to immediate germination of the seeds upon imbibition. Despite the known association between seed coat permeability and embryonic growth potential, the nature of the co-actions between seed coat and embryo growth that determines dormancy is still unclear. A wide range of factors that may potentially disrupt seed coat-imposed dormancy under natural conditions has been identified, with differing implications for seed bank dynamics and seedling emergence patterns (Gama-Arachchige et al., 2012). Chilling plays an important role in providing the stimulus required to overcome dormancy via increases in GA_3 concentration (Bretzloff and Pellett, 1979). Asgari et al. (2015) showed that two-month chilling treatment had the greatest impact on seedling weight of *Nepeta haussknechtii*, *N. menthoides*, *N. cataria*, and *N. crassifoli*. This study aimed to determine treatments that can stimulate and enhance germination of *Bunium luristanicum* seeds.

2. Materials and methods

The mature seeds of *Bunium luristanicum* were collected from Zarand Kerman in central Iran (Lat: 30.8166° N, Long: 56.5729° E) in 2021. After seed collection, the immature and damaged seeds were removed. The seeds were surface sterilized by soaking in 1 % sodium hypochlorite (NaOCl) for 5 min and subsequently, rinsed thoroughly with the sterilized water prior to applying any treatment. Seeds were placed on double layered Wath man No. 1 filter paper moistened with 5 mL of distilled water in sterilized Petri dishes. In each Petri, 25 seeds were sown. Sixteen treatments of breaking dormancy were as follows:

T1: control seeds. T2, T3 and T4, Seeds soaking in distilled water, KNO_3 1 % and GA_3 (500 ppm), respectively for 12 hours. T5 and T6: Seeds were dipped in concentrated sulfuric acid (50 and 75 %), respectively for 3 min. T7 and T8: Seeds were dipped in hot water (70 °C and 90 °C), respectively for 10 min. T9, T10; Seeds were dipped in concentrated sulfuric acid (50 and 75 %), respectively for 3 min, then soaked in a GA_3 500 ppm solution for 12 hours. T11, T12; Seeds were dipped in hot water (70 °C and 90 °C), respectively for 10 min, then soaked in GA_3 500 ppm solution for 12 hours. T13 and T14: Seeds were dipped in concentrated sulfuric acid (50 and 75 %), respectively for 3 min, then KNO_3 0.1 % solution was used for 12 hours. T15 and T16: Seeds were dipped in hot water (70 °C and 90 °C), respectively for 10 min. Then, KNO_3 0.1 % solution was used for 12 hours.

After each treatment, seeds were transferred into the germinator with continuous darkness, a constant temperature of 20 °C, and relative humidity between 70 % and 75 %. Germinated seeds were counted and removed every 24 h for 45 days (Nadjafi et al., 2006). A seed was considered germinated when the tip of the radicle had grown free of the seed coat.

Germination percent, germination rate, and Seed vigor index were measured based on the following equations.

$$\text{Germination}\,\% = n/N \times 100$$

$$\text{Germination rate} = \sum(n_1/t_1 + n_i/t_i)$$

$$\textit{Vigor index} = \text{Germination}\,\% \times \textit{Mean}(\text{shoot length} + \text{root length})$$

Where:
n = the number of germinated seeds,
N = the total number of seeds
n_i = the number of germinated seeds in day t_i.
After the arcsine transformation, data were analyzed of variance using a completely randomized design with five replications and the LSD test was used for means comparison at $P < 0.05$.

3. Results and Discussion

Result of analysis of variance showed significant differences among the 16 treatments for all characters studied (Table 1).

3.1 Germination test

Results showed that the lowest germination percent with value of 13 % obtained in control treatment. Germination percent under application of GA_3 + hot water 70 °C, 90 °C and GA_3 + H_2SO_4 50 % were increased to 82, 79 and 80 %,

Table 1. Effect of seed dormancy breaking treatment on Bunium luristanicum germination and seedling growth

No.	Dormancy breaking treatments	Germination percentage	Abnormal Seedling percentage	Germination rate (no./day)	Root length (mm)	Shoot Length (mm)	Seedling Dry weight (mg)	Vigor index
T1	Control	13 g	1.0 e	0.3 e	18 f	11 e	6.6 f	297 f
T2	Soaking in water (12 h)	15 g	1.0 e	0.4 e	16 f	14 ed	6.4 f	272 f
T3	Soaking in KNO_3 1 % (12 h)	22 f	1.0 e	2.0 c	23 e	13 ed	8.5 e	447 f
T4	Socking in GA_3 (500 ppm 12 h)	38 d	1.0 e	1.6 d	23 e	17 d	10.6 d	1809 e
T5	H_2SO_4 50 % (3 min)	30 e	7.0 c	1.7 d	23 e	19 d	11.9 c	1169 e
T6	H_2SO_4 75 % (3 min)	63 c	14.0 a	2.4 c	23 e	27 c	8.3 e	1782 e
T7	Hot water 70 °C(10 min)	60 c	1.0 e	2.3 c	32 d	25 c	12.3 b	2894 d
T8	Hot water 90 °C(10 min)	58 c	6.0 c	2.9 bc	30 d	33 b	13.2 b	2798 d
T9	GA_3 (500 ppm) + H_2SO_4 (50 %)	80 a	9. b	3.4 b	49 ab	41 a	16.9 a	2844 d
T10	GA_3 (500 ppm) + H_2SO_4 (75 %)	76 ab	14.0 a	4.1 a	43 b	34 b	13.9 b	5789 b
T11	GA_3 (500 ppm) + Hot water (70 °C)	82 a	5.0 d	4.2 a	55 a	38 a	14.9 a	8012 a
T12	GA_3 (500 ppm) + Hot water (90 °C)	79 ab	9.0 b	4.3 a	42 b	32 b	9.9 e	5326 b
T13	KNO_3 (1 %) + H_2SO_4 (50 %)	67 bc	12.0 a	2.8 bc	46 b	40 a	14.5 b	4265 c
T14	KNO_3 + H_2SO_4 (75 %)	74 b	1.0 e	3.2 b	38 c	32 b	13.1 c	4258 c
T15	KNO_3 + Hot water (70 °C)	76 b	1.0 e	3.2 b	42 b	38 a	14.3 b	4402 c
T16	KNO_3 + Hot water (90 °C)	69 b	8.0 b	3.2 b	40 b	36 a	12.1 b	4241 c
T1	Means of square (MS)	1243.1**	11.9**	8.2**	3.5**	0.523**	7.3**	218**

** Significant at the 0.01 probability level; Means of column with the same letter are not significantly different.

respectively. These results showed that *B. luristanicum* seed dormancy was influenced by physiological and physical factors. The application of GA_3, H_2SO_4 50 %, H_2SO_4 75 %, hot water 70 °C, and hot water 90 °C increased germination percent by 38, 30, 63, 60, and 58 %, respectively. It was concluded that the seed dormancy *B. luristanicum* was more affected by physical factors because the scratching treatments were more effective than the application of GA_3 or KNO_3 alone (Table 1). Using combined treatments of GA_3 and scratching the seed coat, the highest germination percent with values 80, 67 and 79 % was obtained by application of (KNO_3 + H_2SO_4 70 %), (KNO_3 + Hot water 70 °C), and (KNO_3 + Hot water 90 °C), respectively. The effect of GA_3 on seed germination of *B. luristanicum* was non-deep physiological seed dormancy as Zhou and Bao (2011) came to the same conclusion.

3.2 Abnormal seedling

Result showed that GA_3 and KNO_3 with average values of 14 %, 14 %, and 12 % had the highest abnormal seedlings, respectively. Application of H_2SO_4 50 % as well as hot water 90 °C alone or in combination with other treatments or GA_3 and KNO_3 produced lower abnormal seedlings. Nabaee et al. (2013) reported the combination of KNO_3, with hot water to breaking dormancy of *Arctium lappa* seeds. In contrast, application of higher concentrations of H_2SO_4 had increased the number of abnormal seedlings (L. et al., 2005).

3.3 Germination rate

Results showed that the highest seed germination rate with average values of 4.3, 4.2 and 4.1 seeds per day were obtained under the application of GA_3 + hot water 90 °C, GA_3 + Hot water 70 °C and GA_3 + H_2SO_4 75 %, respectively (Table 1). Control treatment and seed soaking in water had the lowest germination rate (0.3 and 0.4) seeds per day, respectively. Hot water 70 °C, hot water 90 °C, H_2SO_4 50 %, H_2SO_4 75 % and GA_3 also increased the germination rate to 2.3, 2.9, 1.7, 2.4, and 1.6 seeds per day, respectively. These results showed that scratching the seed coat with H_2SO_4 coupled with GA_3 increases seed germination rate (Table 1). The present study showed a synergistic effect of GA_3 and KNO_3 solution on promoting seed germination rate of *B. luristanicum*. Similar synergistic effects had been reported for *Sabal palmetto* (Dewir et al., 2011) and *Elaeocarpus prunifolius* (Iralu and Upadhaya, 2018).

3.4 Root and shoot length

The maximum root length of 55 and 49 mm was observed using GA_3 + hot water 70 °C and GA_3 + H_2SO_4 50 %, respectively. The application of hot water at 70 and 90 °C alone had a much longer root length than the application of H_2SO_4 50 % and 75 %, GA_3, or KNO_3 (Table 1). The lowest root length was observed in the control treatment and seed soaking in water (18 and 16 mm), respectively. The lowest shoot length was observed in the control treatment, soaking the seeds in water and KNO_3 (11, 14, and 13 mm, respectively). Treated seeds with GA_3 + H_2SO_4 50 %, GA_3 + hot water 70 °C, and KNO_3 + hot water 70 °C increased the shoot length to 41, 38, and 38 mm, respectively (Table 1).

3.5 Seedling dry weight

In this study, the application of GA_3 and KNO_3 increased the seedlings dry weight to 10.6 and 8.5 mg, respectively, which indicates more significant effect of GA_3 on *B. luristanicum* seedling growth. Also, the shoot length in *B. luristanicum* was significantly affected by GA_3 more than the application of KNO_3 (Table 1). Application of physical treatments such as GA_3 + H_2SO_4 (50 %) and GA_3 + Hot water (70 °C) with average values of 16.9 and 14.9 mg produced heavier seedling, respectively, but hot water 90 °C or high concentrated sulfuric acid produced the damaged seedling.

3.6 Vigor index

The highest seed vigor index was observed in seeds treated with GA_3 + hot water 70 °C; however, the lowest seed vigor index was recorded in control treatments, seed soaking in water and control. GA_3 + hot water 70 °C had increased seed vigor index followed by GA_3 + H_2SO_4 50 % and 75 %, GA_3 + hot water 90 °C, KNO_3 + hot water 70 °C (Table 1).

Seed vigor index indicates seed potential for germination, seedling growth, and tolerance to adverse environmental conditions during germination and it is an essential indicator during germination, influenced by various factors such as genetics, environmental conditions in the seed development period and seed germination improvement treatments (Hu et al., 2018). Therefore, it can be expected that seed vigor will increase via seed breaking dormancy treatments which will lead to increased germination rate, seedling growth and germination percent. It was concluded that for *B. luristanicum* seeds, GA_3 with hot water stimulated seed germination and had more significant effect than the other treatments applied in this study. Tavili et al. (2014) showed that sulfuric acid had higher effect in breaking dormancy, but its application in a vast scale is not easy; therefore, the hot water could be considered as the substitution treatment.

4. Conclusions

Considering the results of the present research, it was concluded that seed immersion in GA_3 + hot water 70 °C was the best treatment for both improving the germination factor and producing healthy seedlings. Although treatments like GA_3 + hot water 90 °C, and GA_3 + H_2SO_4 75 % also produced the highest germination percent, they led to the damage to the seedling structure, decrease in seedling weight, increase in the percentage of abnormal seedlings and finally decrease the seed vigor index. Due to the ease of using the hot water treatment of GA_3, it was recommended that using GA_3 500 ppm + scratching the seed coat with hot water 70 °C for 10 minutes could improve the germination of *B. luristanicum* seeds.

Ethical approval:
This manuscript does not report on or involve the use of any animal or human data or tissue. So the

ethical approval does not applicable.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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