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EFFECTS OF VARIOUS DORMANCY BREAKING TREATMENT ON SEED GERMINATION IN *Sclerorhachis Leptoclada* BOISS: AN ENDANGERED MEDICINAL PLANT IN ARID AREA

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The aim of this study was to investigate the effect of pre-sowing treatments and temperature on *Sclerorhachis leptoclada* Boiss. seed germination. The following treatments were used), hot water (soaking in water at 70°C and 90°C for 5 second), Moist Chilling (Soaking in water at 2°C for 7,14 and 21day with the temperature gradually falling to room temperature), scarification by 0.2% potassium nitrate (soaking for 24 h and 48 h) and Gibberellic acid (GA: at concentrations of 250, 500 and 750 ppm soaking for 24 h and 48 h) and Distilled Water (control). The percentage of germination, germination rate, germination start (GS), mean germination time (MGT), and germination vigor index were determined as germination indices. Root and shoot length, root fresh weight, root dry weight, shoot fresh weight, shoot dry weight and seedling dry weight were evaluated as growth factors at the end of the incubation period. Second experiment was carried out to study the effect of temperature on seed germination. It was observed that both the physical and chemical scarification methods were effective in enhancing *S. leptoclada* seed germination and growth. The highest germination rate, percentage of

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germination and germination vigor index was observed with Moist Chilling (Soaking in water at 2°C for 14 day), 0.2% potassium nitrate (soaking for 48h) and GA (at concentration of 250 ppm soaking for 48h) in compare with control. Also the highest growth factors were found in Moist Chilling (Soaking in water at 2°C for 7day) treated seeds and the lowest occurred in hot water treatments. The other results showed that higher temperature was more effective lower temperature for seed germination.

Key words: germination, pretreatment, seed dormancy, scarification, *Sclerorhachis leptoclada* Boiss

INTRODUCTION

Seeds are an important part in the development of plants but Seeds of many plants do not germinate even under the most favorable conditions (URGENÇ and ÇEPEL, 2001). Seed germination can be controlled by many factors like natural germination inhibitors and Poor seed germination is one of the main problems of threatened medicinal plants production within the native environment.

Sclerorhachis leptoclada Boiss of the Asteraceae family is an endemic herbaceous plant in Iran (MOZAFFARIAN, 1996) and found in the most arid parts of highlands in eastern Iran (RICHINGER, 1982). Recent pharmacological studies have confirmed some medicinal properties of *S. leptoclada* including anti-inflammatory (KALLIVALAPPIL and KUTTAN, 2017), antimicrobial (GHADERI and SONBOLI, 2018), antioxidant (BABOTA *et al*., 2018), anti-protozoa (GARCIA *et al*., 2017), healing activities (OZBILGIN *et al*., 2018) and treatment of wounds (PARENTE *et al*., 2012; NAYAK *et al.*, 2014). This species is mostly distributed by seeds but its germination rate is low. Great harvesting of wild plants, restricted distribution areas, and a lack of cultivation and domestication, are the main cause of *S. leptoclada* is listed as an endangered plant (JALALI and JAMZAD, 1999). As a consequence of dormancy, the germination percentage of *S. leptoclada* is very low. Preliminary work showed that one of the main restrictions of this plant is low seed germination within the native environment, which could be a result of dormancy induced by a mucilaginous material. Dormancy is determined by both the morphological and physiological properties of seeds, which should be considered when studying treatments for improving seed germination (NIKOLAEVA, 2004). In order to accelerate breaking seed dormancy and increasing seed germination have been assessed by different researchers (FATTAHI *et al*., 2011; SHINDE and CHAVAN, 2017; MILADINOV *et al*., 2018; BILLAH *et al*., 2015; WAJID *et al*., 2018; DEGROOT *et al*., 2018; BURROWS *et al*., 2018; OGUNROTIMI and KAYODE, 2018). Moist Chilling, scarification, and treatments with gibberellic acid and potassium nitrate are the standard procedures used to enhance seed germination of dormant seeds. Although scarification (by physical and chemical means) and treatment with plant growth regulators are known to influence seed germination in several species (PANDEY *et al*., 2000; BAES *et al*., 2002; EMONGOR *et al*., 2004; ZIDA *et al*., 2005; TANAKA-ODA *et al*., 2009), such treatments have not been reported for *S. leptoclada* despite its high medicinal value. Thus, the aim of this work was to study the effect of several physical, chemical and hormonal treatments on *S. leptoclada* seeds and establish a suitable method to overcome their low germination rate. The effect of temperature condition on seed germination was also investigated to facilitate domestication of this endangered plant.

MATERIAL AND METHOD

Seed Source

The mature seeds of *S. leptoclada* were collected from Northeast of Iran (Lat 33° N; Lon 58° 37/ E) in 2017. The seeds were surface sterilized by soaking in 0.2% sodium hypochlorite (NaOCl) for 15sec and subsequently rinsed thoroughly with sterilized water prior to applying any treatment. Seeds were placed on double layered Wathman No.1 filter paper moistened with 5ml of distilled water in sterilized Petri dishes. The pre-sowing treatments varied as a consequence of differences in the seed germination for each as follows:

Pre-sowing and scarification Treatment

Based on literature and low seed germination of *S. leptoclada*, the following pre-sowing treatments were used for overcoming seed dormancy: The first factorial were: Distilled Water (Soaking in Distilled water for 24 and 48 h with the temperature gradually falling to room temperature), hot water (soaking in water at 70°C and 90°C for 5 sec), Moist Chilling (Soaking in water at 2°C for 7,14 and 21day with the temperature gradually falling to room temperature), scarification by 0.2% potassium nitrate (soaking for 24 h and 48 h) and Gibberellic acid (GA: at concentrations of 250, 500 and 750 ppm soaking for 24 h and 48 h). The second factor was temperature (10°C and 15°C). All treatments were carried out in two-factorial based on a completely randomized design with four replications and the results were compared with the control, which did not undergo any treatment to induce seed germination.

Seed Germination Conditions

A total of 15 pre-sowing treatments were performed in this experiment, as described above. Control and treated seeds were placed in plastic pots filled with perlite. Each germination treatment, including the control, was performed with four replications. In each replicate, 20 seeds were used, divided equally among 4 pots, giving a total of 80 seeds in each pre-sowing treatment. The pots were placed in greenhouse at 8h night and 16h day with 70% humidity. The number of germinated seeds was counted daily by observing the rot and shoot emerging from the bed surface.

Data Analysis

The indices used to evaluate germination were the following:

1- Percentage of Germination: = (n/N), 100 n = the total number of seeds germinated and N = the number of seeds used at the beginning of the experiment.
2- Germination Start (GS) was the time between seed sowing and the beginning of germination.
3- the Germination Rate (GR) was calculated using the formula (WIESE and BINNING, 1987).
4- Mean Germination Time (MGT) was calculated using the equation (ELLIS and ROBERTS, 1981).
5- The Germination Vigor Index (GVI): [seedling length (cm) × germination percentage] (MORADI DEZFULI *et al*., 2008).

Plant growth in the incubation period was evaluated by root length (cm), shoot length (cm), root fresh weight(g), root dry weight(g), shoot fresh weight(g), shoot dry weight(g) and seedling dry weight (g) at the end of the period.

Statistical Analyses

The data were subjected to analysis of variance using SAS 9.2 for a completely randomized design and significant differences were determined using Duncan's Multiple Range Test (DMRT) at 0.01 probability.

RESULTS

The effect of pre-sowing treatment on seed germination

Percentage of Germination

In the first stage, D1[1]resulted in a significantly higher percentage of germination (18%) than D2[2]. Similarly, Moist Chilling for 14 and 21 days improved seed germination compared with the control. GA treatment was most effective at 250 ppm on all recorded days, while 500 and 750 ppm GA did not produce any significant difference in the germination percentage. Finally, hot water blocked the capacity of the treated seeds for germination (Table1).

Germination Start

Germination start (GS) varied significantly between treatments at t $P < 0.01$ level. GS in seeds treated with Moist Chilling for 14 and 21day and 0.2% potassium nitrate for 48h (1), 0.2% potassium nitrate for 24h and GA at concentrations of 250 ppm for 48h (2) and GA at concentrations of 500 ppm for 24h (3) was earlier than in the control (distilled water for 48h) (4). The other treatments also showed a lower GS index with little difference among them (Table1).

Germination Rate

The germination rate ($p < 0.01$) differed significantly between the pre-sowing treatments, the highest being observed with p14[3](100%) and p6[4](80%). Increasing temperature from 5°C to 10°C improved GR in seed treated with Moist Chilling (Soaking in water at 2°C for 14 day) (Table 1).

Mean Germination Time

Mean germination time was significantly affected by different pre-sowing treatments at $P < 0.01$. The highest MGT was observed in Moist Chilling for 14 days and seed treated with hot water showed the lowest MGT. GA (at concentration 500 ppm and 750 ppm) gave a lower MGT than the control. The other treatments had not shown significant difference in compare with control (Table 1).

[1] Pre-sowing-treatment: Temperature (15°C)
[2] Pre-sowing-treatment: Temperature (10°C)
[3] Pre-sowing-treatment: Moist Chilling (Soaking in water at 2°C for 14 day)
[4] Pre-sowing-treatment: 0.2% potassium nitrate (soaking for 48 h)

Germination Vigor Index

VI was significantly influenced by various treatments at ((P < 0.01). The highest VI was found in Moist Chilling treated seeds and the lowest occurred in hot water treatment. Temperature and Moist Chilling treatments gave a higher VI than the control (Table 1).

Table 1. Effects of different treatments on seed germination indices

Treatments	Duration	POG	GS	GR	MGT	GVI
control (Distilled Water)	24h	55 $^{f-g}$	6	19.60 $^{f-h}$	5.237 ^{cd}	2.713 ^{de}
Control (Distilled Water)	48h	57 $^{e-g}$	4	21.81 ^{fg}	5.75 ^{cd}	2.914 ^{de}
Hot water (at 70°c)	5 sec	0 ^{j}	10	0.000 ^{k}	0.000 ^{g}	0.000 ^{g}
Hot water (at 90°c)	5 sec	0 ^{j}	-	0.000 ^{k}	0.000 ^{g}	0.000 ^{g}
0.2% potassium nitrate	24h	69.50 ^{cd}	2	29.29 ^{de}	6.178 ^{c}	4.853 ^{c}
0.2% potassium nitrate	48h	76.50^{Bc}	1	38.11 ^{c}	7.829 ^{b}	6.034 ^{b}
GA 250ppm	24h	63.50 $^{d-f}$	4	24.45 ^{ef}	6.119 ^{c}	3.236 ^{d}
GA 250ppm	48h	73. 50 ^{c}	2	30.79 ^{d}	7.230 ^{b}	4.197 ^{c}
GA 500ppm	24h	52 ^{g}	3	16.33 ^{gh}	4.823 ^{d}	2.134 ^{e}
GA 500ppm	48h	32 ^{h}	5	9.275 ^{ij}	2.936 ^{e}	1.239 ^{f}
GA 750ppm	24h	26 ^{h}	8	5.378 ^{jk}	2.052 ^{ef}	0.623 ^{fg}
GA 750ppm	48h	14 ^{i}	12	3.172 ^{k}	1.122 ^{f}	0.348 ^{fg}
Moist Chilling (Soaking in water at 2°C)	7day	64 ^{de}	5	14.46 ^{hi}	5.223 ^{cd}	2.336 ^{de}
Moist Chilling (Soaking in water at 2°C)	14day	92 ^{a}	1	48.08 ^{b}	9.314 ^{a}	6.598 ^{b}
Moist Chilling (Soaking in water at 2°C)	21day	84 ^{ab}	1	58.94 ^{a}	8.846 ^{a}	9.426 ^{a}
Prob.		***	***	***	***	***

POG: Percentage Of Germination, GR: Germinatio Rate, MGT: Mean Germination Time, GVI: Germination Vigor Index. a-k: Means by at least one letter in common are not significantly different probably level using Duncan's Multiple Range Test (DMRT) at 0.05 probability. ***Significant at (P < 0.001).

Evaluation of seedlings at the end of incubation

Root Length

Analysis of variance showed significant difference (P < 0.01) in root length between treatments. The maximum root length (17.2 cm) was recorded in seed treated with 0.2% potassium nitrate (soaking for 48 h) and the minimum (0 cm) was found with hot water. Treatment with high temperature also resulted in shorter roots. The results of the other treatments did not differ significantly from the control (Table 2).

Shoot Length

The differences in shoot length between various treatments were statistically significant at (P < 0.01). The highest shoot length was recorded in seedlings treated with Moist Chilling

(Soaking in water at 2°C for 7 day) and 15°C temperature (21.7mm), and the lowest with GA (at concentration 750 ppm for 48 h) treatment (11.63mm). Also 0.2% potassium nitrate treated seed had shown higher shoot length in compare with control (Table 2).

Root Fresh Weight

Root Fresh Weight was significantly different between various treatments at ($P < 0.01$). The highest Root Fresh Weight was observed with GA at concentration 250 ppm (0.096g) and 0.2% potassium nitrate (0.0.86) and the lowest with Moist Chilling for 21day treatment (0.031g).

Root Dry Weight

Analysis of variance showed significant differences ($P < 0.01$) in Root Dry Weight between treatments. The highest Root Dry Weight was observed with 0.2% potassium nitrate and GA at concentration 250 ppm (Table2).

Shoot Fresh Weight

Shoot Fresh Weight was significantly influenced by various treatments at ($P < 0.01$). The highest Shoot Fresh Weight was found in 0.2% potassium nitrate and GA at concentration 250 ppm treated seeds (Table2).

Table 2. Effect of different treatments on seedling growth factors at the end of incubation time

Treatments	Duration	RL (cm)	ShL (cm)	RFW (g)	RDW (g)	SHFW (g)	SHDW (g)	SDW (g)
control (Distilled Water)	24h	11.75 cd	15.99 bc	0.035 d-f	0.0013 a	0.064	0.0022 a	0.0036 a
control (Distilled Water)	48h	12.65 bc	13.77 de	0.045 b-d	0.0018 a	0.055 bc	0.0022 a	0.0037 a
Hot water (at 70°c)	5 sec	0.000 g	0.000 g	0.000 h	0.000 a	0.000 g	0.000 a	0.000 a
Hot water (at 90°c)	5 sec	0.000 g	0.000 g	0.000 h	0.000 a	0.000 g	0.000 a	0.000 a
0.2% potassium nitrate	24h	15 ab	16.16 bc	0.062 a	0.0020 a	0.062 ab	0.0024 a	0.0045 a
0.2% potassium nitrate	48h	17.17 a	15.31 cd	0.049 bc	0.0020 a	0.069 a	0.0027 a	0.0048 a
GA 250ppm	24h	11.29 c-e	15.29 cd	0.066 a	0.0023 a	0.058 ab	0.0025 a	0.0048 a
GA 250ppm	48h	11.46 cd	15.74 c	0.051 b	0.0021 a	0.059 ab	0.0026 a	0.0048 a
GA 500ppm	24h	9.393 d-f	15.56 cd	0.039 c-e	0.0014 a	0.058 ab	0.0028 a	0.0042 a
GA 500ppm	48h	8.449 c-e	14.90 c-e	0.026 f	0.0012 a	0.037 d	0.0018 a	0.0030 a
GA 750ppm	24h	11.13 ef	13.43 e	0.011 g	0.0003 a	0.027 e	0.0011 a	0.0014 a
GA 750ppm	48h	6.683 f	11.63 f	0.013 g	0.0002 a	0.016 f	0.0007 a	0.0001 a
Moist Chilling (Soaking in water at 2°C)	7day	13.13 bc	17.60 ab	0.037 de	0.0007 a	0.047 c	0.0013 a	0.0021 a
Moist Chilling (Soaking in water at 2°C)	14day	11.20 c-e	16.10 bc	0.035 d-f	0.0012 a	0.053 bc	0.0020 a	0.0033 a
Moist Chilling (Soaking in water at 2°C)	21day	13.27 bc	18.07 a	0.031 ef	0.0011 a	0.062 ab	0.0021 a	0.0033 a
Prob.		***	***	***	***	***	***	***

a-k: Means by at least one letter in common are not significantly different probably level using Duncan's Multiple Range Test (DMRT) at 0.05 probability. ***Significant at ($P < 0.001$).

Shoot Dry Weight

Shoot Dry Weight was significantly influenced by various treatments at ($P < 0.01$). The highest Shoot Dry Weight was found in 0.2% potassium nitrate and GA at concentration 250 ppm treated seeds (Table2).

Seedling Dry Weight

Analysis of variance showed significant differences ($P < 0.01$) in Seedling Dry Weight between treatments. The maximum Seedling Dry Weight (0.0074g) was recorded in seeds treated with 10℃ temperature and GA at concentration 250 ppm (0.0071g) (Table2).

DISCUSSION

As the medicinally valuable Iranian endemic *S. leptoclada* is currently an endangered species (JALALI and JAMZAD, 1999), its domestication is of special urgency. The first step of a domestication program is to boost plant germination. In the present work, although *S. leptoclada* seeds were collected from wild sources, the results showed they had low germination potential and It seems that the use of pre-sowing treatments were able to increase the seed germination percentage. It has been observed that GA inhibits the germination start in *S. leptoclada* and can cause a high POG in the initial days of incubation. The result is confirmed that the GA is well-known endogenous dormancy-breaking agents (REHMAN and PARK, 2000; TAKANO, 1993; THOMPSON, 1969; CHANG *et al*., 2009) and has significant differences in the germination of GA treated seeds compared with the control. At the low concentration of GA (250 ppm) the GS even increased.

No germinated seeds were found after the treatment with hot water, as they were probably killed by the high temperature, similar results being reported by (YÜCEL, 2000; YÜCEL and YILMAZ, 2009). Although it has been observed that Moist Chilling inhibits both the percentage and rate of germination in *S. leptoclada* (PEREZ-GARCIA and GONZALEZ-BENITO, 2006; VELDHUIZEN and KNIGHT, 2006), we found that both Moist Chilling and scarification by 0.2% potassium nitrate can cause a high percentage of germination in the initial days of incubation. Similar results were reported in previous studies for the species of *Foeniculum vulgar* L. (SOLTANIPOOR *et al*., 2009), *Allyssum homalocarpum* (GANJALI and AJORLO, 2014), *Teucrium polium* (KOOCHAKI and AZIZI, 2005), *Stevia rebaudiana* (LIOPA-TSAKALIDI *et al*., 2012), *Citrullus colocynthis* (SABERI *et al*., 2011), walnut (QURESHI *et al*., 2016), *Phoenix dactylifera L.* (MUHAMMAD *et al*., 2017), *Brassica tournefortii* (MAHAJAN *et al*., 2018), Kiwifruit (ZHANG *et al*., 2018), *Elaeocarpus serratus* L. (RAJI and SIRIL, 2018), Rice seed (SHIRATSUCHI *et al*., 2017), Apple seed (GORNIK *et al*., 2018), *Pistacia khinjuk* (ACAR *et al*., 2017; JIA *et al*. 2020; BI *et al*. 2021) and *Sterculia urens Roxb* (SUBNASHINI-DEVI *et al*., 2012; SI *et al*., 2020;).

Evaluation of growth factors (the length of shoots and roots) showed that they were all improved by Moist Chilling and 0.2% potassium nitrate. Since the germination start is extremely important for the rapid propagation of endangered plants. Thus, Fast germination and growth are important in domestication programs and weed control. Our results showed that the Moist Chilling and scarification by 0.2% potassium nitrate were able to improve the GS in seeds by the begin of the incubation period, probably due to the high germination rate they induced. This technique has become a common seed treatment that can increase seed indices and growth

factors in *S. leptoclada*, mainly under unfavorable environmental conditions. Positive effect of 0.2% potassium nitrate could be due to its role in balancing hormonal portion within seed which in turn results in germination inhibitors ratio like ABA (Abscisic Acid). The seeds of most Mediterranean and desert species have dormancy characteristics or structural properties that prevent immediate germination of at least a proportion of the seeds (THANOS *et al*., 1989; JURADO and WESTOBY, 1992; GUTTERMAN, 1993; ROKICH *et al*., 1995; ZHU, ET AL., 2021; Zhao *et al.* 2021; YIN, *et al.* 2021; MA, *et al.* 2021; PENG, *et al.* 2021).

CONCLUSION

According to the obtained results, it is suggested that seeds are exposed to Moist Chilling for 7day and 0.2% potassium nitrate in 15$^{\circ c}$ temperature, which results in overcoming seed dormancy of *S. leptoclada*. Scarification by 0.2% potassium nitrate is a simple, safe, and reliable way to improve germination rates in *Sclerorhachis* species. Our results also showed that this plant has a low capacity for germination (35%) and temperature can be an important factor in promoting its rapid germination. It can be concluded that for *S.eptoclada* Moist Chilling alone or in combination with 0.2% potassium nitrate stimulates seed germination and has a larger effect than the other treatments applied in this study. Future studies are necessary to fully understand what other factors may affect the domestication of this valuable medicinal plant. The results obtained will be useful in carrying out medicinal plants improvement and plantings of *S. leptoclada* for local medicine and industrial production. Rapid germination is also essential for reclamation of desert and this information could ultimately help in the sustainable development of the arid zones.

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EFEKAT RAZLIČITIH TRETMANA DORMATNOSTI NA KLIJANJE SEMENA *Sclerorhachis Leptoclada* BOISS: MEDICINSKE BILJKE U SUŠNIM OBLASTIMA

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Izvod

Cilj ovog istraživanja bio je da se ispita efekat predsetvenih tretmana i temperature na klijavost semena kod *Sclerorhachis leptoclada Boiss*. Korišćeni su sledeći tretmani: topla voda (potapanje u vodi na 70°C i 90°C u trajanju od 5 sekundi), vlažno hlađenje (potapanje u vodi na 2°C tokom 7,14 i 21 dan sa postepenim snižavanjem temperature na sobnu temperaturu), skarifikacija 0,2% kalijum nitrata (kvašenje 24 h i 48 h) i Giberelinska kiselina (GA: u koncentracijama od 250, 500 i 750 ppm namakanje 24 h i 48 h) i destilovana voda (kontrola)). Procenat klijanja, stopa klijanja, početak klijanja (GS), srednje vreme klijanja (MGT) i indeks snage klijanja određeni su kao indeksi klijanja. Dužina korena i izdanaka, sveža masa korena, suva masa korena, sveža težina izdanaka, suva težina izdanaka i suva težina sadnica su procenjeni kao faktori rasta na kraju perioda inkubacije. Drugi eksperiment je sproveden radi proučavanja uticaja temperature na klijavost semena. Primećeno je da su i fizičke i hemijske metode skarifikacije bile efikasne u poboljšanju klijanja i rasta semena *S. leptoclada*. Najveća stopa klijanja, procenat klijanja i indeks snage klijanja primećeni su kod vlažnog hlađenja (potapanje u vodi na 2°C tokom 14 dana), 0,2% kalijum nitrata (kvašenje 48h) i GA (pri koncentraciji od 250 ppm, namakanje 48h). u poređenju sa kontrolom. Takođe, najveći faktori rasta su pronađeni u semenima tretiranim vlažnim hlađenjem (kvašenje u vodi na 2◦C tokom 7 dana), a najmanji su se desili u tretmanima toplom vodom. Ostali rezultati su pokazali da je viša temperatura efikasnija od niže temperature za klijanje semena.

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