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# Seed germination improvement of *Satureja khuzistanica* and *S. rechingeri* (*Lamiaceae*) as valuable endemic medicinal species from Iran

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## Abstract

The objective of this study was to enhance seed germination of two valuable and endemic medicinal species, *Satureja khuzistanica* and *S. rechingeri,* which commonly have low percentage and rate of germination. For this purpose, a factorial experiment in randomized complete design (RCD) with three replications each consisted of 20 seeds, was performed under laboratory conditions. The efficacy of different seed treatments including various concentrations of $GA_3$ (250, 350 and 500 ppm), cold stratification (at 5 ºC for 7 and 14 days), soaking (24h in water), $KNO_3$ (1 and 3%), alone or in combination with each other, on seed germination of both species was studied. The highest germination percentage and germination rate was observed in both species at low concentration of $GA_3$ (250 ppm), followed by chilling stratification at 5 ºC for 7 days. Increase in $GA_3$ concentration or period of cold stratification decreased the percentage and rate of germination in both species. Also soaking in water for 24h significantly enhanced germination percentage and germination rate of both species compare to control.

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**Introduction**

The genus *Satureja*, with common Persian name of "Marzeh", belongs to the family of Lamiaceae, subfamily Nepetoideae that is represented in Iran by more than fourteen species of which nine as *S. khuzistanica* Jamzad and *S. rechingeri* Jamzad are endemic (Rechinger., 1982; Jamzad., 1994).

Both *S. khuzistanica* Jamzad and *S. rechingeri* are strongly scented plants and are used as herbal tea and for the therapeutic value as analgesic and antiseptic in traditional medicine. Their chemical profile is very similar and carvacrol is the major constituent of their essential oils, while their extracts are rich in phenolic acids and flavonoids (Farsam *et al.*, 2004; Hadian *et al.*, 2009, 2010, 2011). During the last decade, pharmacological properties of *S. khuzistanica* have been extensively studied. Antioxidant, antimicrobial, antidiabetes, anti-biofilm, anti-inflammatory, anti-nociceptive, and anti-hyperlipidemic and reproductive stimulation effects of *S. khuzistanica* have been proved (Abdollahi *et al.*, 2003; Rezvanfar *et al.*, 2008; Rezvanfar *et al.*, 2010; Behravan *et al*, 2004; Basiri *et al.*, 2007).

*Satureja khuzistanica* and *S. rechingeri* are growing wild on calcareous rocky soils in the arid areas of south west of Iran (Hadian, 2009). Increasing consumption and high demand of pharmaceutical industry for the raw material of *S. khuzistanica* and *S. rechingeri* leads to over collection of the plants from the nature. In the other hand, extinction danger of these valuable species due to more harvesting from the nature has made it necessary to introduce these medicinal species to the farming systems. Recently, a domestication program has been started for cultivation and commercial production of *S. khuzistanica* and *S. rechingeri* in the agricultural systems (Hadian *et al.*, 2011).

Seed dormancy is an important limiting factor in exploitation of an economically important species to its fullest (Gupta, 2011). Successful establishment of plants largely depends on successful germination (Gorai *et al.*, 2007). A lot of medicinal plants fail to show good germination under artificial cultivation which make their propagation difficult (Gupta, 2003). Preliminary studies revealed that the seed germination of *S. khuzistanica* and *S. rechingeri* is weak and little information on seed dormancy-breaking and germination requirements of these species are available. For this purpose, it would be necessary to find a suitable method for seed germination improvement of these species. The aim of the present study was to determine the effect of different seed treatments which are able to stimulate and enhance the germination of both *S. khuzistanica* and *S. rechingeri* seeds.

**Materials and methods**

*Seed materials*

The mature seeds were collected during November from cultivated stocks of *S. khuzistanica* and *S. rechingeri* in the field of Kashkan Research Station, Khorraman Pharmaceutical Company, Khorram-Abad, in the West of Iran (33° 29´N, 48° 21´ E).

*Seed treatments*

After collection, immature and broken seeds or unwanted materials were removed and seed viability was determined using Tetrazolium chloride (TTC, Merck, Darmstadt, Germany) method. The seeds were surface sterilized by soaking in 3% sodium hypochlorite (NaOCl) for 5 min and subsequently rinsed thoroughly with sterilized water prior to applying any treatment.

A factorial experiment, based on a completely randomized design with three replications, each consisted of 20 seeds, was used. The seeds were subjected to different treatments including i) soaking for 48h in gibberellic acid, GA3 (250, 350 and 500 ppm), ii) cold stratification (keep the moisturized seeds at 5 °C for 7 and 14 days), soaking the seeds for 24h in water), and iii) soaking for 24h in KNO3 solution (1 and 3%). Combinations of the treatments were also considered as below:

$GA_3$ and cold stratification: seeds were soaked in concentrations of 100, 250, and 350 ppm $GA_3$ for 24h

and then washed within distilled water and kept at 5 ˚C for periods of 7 and 14 days.

$GA_3$ and $KNO_3$: seeds were soaked in $KNO_3$ (1% and 3%) and $GA_3$ (250, 350, 500 ppm) respectively, for 5 h and then washed thoroughly by distilled water, before transferring to the germination test process. No treated seeds were considered as control.

After each treatment, seeds were placed on double layered Wathman No.1 filter paper moistened with 5ml of distilled water in sterilized Petri dishes and transferred to germinators with temperature of 24 ˚C and relative humidity of 70-75%.

*Data collection and analysis*

Germinated seeds were counted and removed each 24h for 16 days. A seed was considered as germinated seed when the tip of the radicle had grown free of the seed coat (Auld *et al*, 1988). Distilled water equal to the mean water loss from dishes was added every day throughout the germination period.

Final germination percentages, germination rate, mean germination time (MGT) were calculated according to the following formulas respectively (Olmez *et al*., 2007, Nadjafi *et al*., 2006 and Scott *et al*., 1984) when no further germination took place for several days:

$$\text{Germination percentage} = \frac{\text{number of germinating seeds}}{\text{number of viable seeds initiated}} \times 100 \quad \text{(Eq. 1)}$$

$$\text{Germination rate} = \sum_{n=1}^{14} (\text{number germinating since } n-1)/n \quad \text{(Eq. 2)}$$

Where, n is the days of incubation.

$$MGT = \sum nd/N \quad \text{(Eq. 3)}$$

Where *d* is the number of days from beginning of the experiment, *n* is the number of seeds germinated on day i and N is the total number of germinated seeds. After Arcsine transformation, data were analyzed with SAS (2001) and the Duncan multiple range test was used to detect significant differences among the treatments with $P<0.05$.

**Results and discussion**

The Tetrazolium test indicated that seeds of *S. khuzistanica* and *S. rechingeri* had a viability of 86% and 92%, respectively. Analysis of variance (Table 1.) showed a significant difference between species in germination rate and germination percentage but the mean germination time was not significantly affected by species. As shown in Table 1, the results revealed that pre-germination treatments significantly affected seed germination parameters such as germination percentage, germination rate and mean of germination time in both species.

**Table 1.** Analysis of variance of different pre-treatments on seed germination of *Satureja khuzistanica* and *S. rechingeri.*

| Source of variation | DF | Mean Square | | |
|---|---|---|---|---|
| | | GP | GR | MTG |
| species | 1 | 350.000 * | 0.773 * | 8.316 ns |
| Treatments | 20 | 1602.341** | 2.23** | 10.465 ** |
| Treatments × species | 20 | 147.500 ** | 0.245 ** | 4.762 ns |
| Error | 84 | 52.579 | 0.086 | 3.892 |

DF, degrees of freedom; GP, germination percentage; GR, germination rate; MGT, mean germination time; ns, no significant differences; * significantly ($P<0.05$); ** significantly ($P < 0.01$).

The interaction effect of species and pre-germination treatments, were significant on germination percentage and germination rate but mean of germination time was not affected. Our results also showed that germination rate and germination percentage of treated seeds of *S. rechingeri* were higher than *S. khuzistanica (*Table 2.*)*. Pre-germination treatment of seeds with $GA_3$ stimulated the germination of both species.

**Table 2.** Germination percentage (GP) and germination rate (GR) in two species of *Satureja khuzistanica* and *S. rechingeri.*

| Species | GP | GR |
|---|---|---|
| *S. khuzistanica* | 21.827 b | 0.71 b |
| *S. rechingeri* | 25.159 a | 0.87 a |

This response was dependent on the concentration of applied $GA_3$. At lower concentrations, germination of both species was higher. The highest germination percentage and germination rate obtained at $GA_3$ concentration of 250 ppm *(*Table 3.).

**Table 3.** The effect of different pre-germination treatments on seed germination of *S. khuzistanica* and *S. rechingeri.*

| pre-germination treatments | GP | GR | MGT |
|---|---|---|---|
| C | 25.83 d-f | 0.921 c-e | 6.098 ab |
| $CS_1$ | 55 b | 1.692 b | 7.144 a |
| $CS_2$ | 24.17 d-g | 0.826 d-f | 6.392 a |
| $K_1$ | 26.67 d-f | 0.816 d-f | 7.07 a |
| $K_2$ | 17.50 f-h | 0.566 e-i | 6.69 a |
| $GB_1$ | 72.5 a | 2.825 a | 6.345 a |
| $GB_2$ | 32.5 cd | 1.102 cd | 6.72 a |
| $GB_3$ | 30 de | 1.16 cd | 6.512 a |
| S | 39.17 c | 1.277 bc | 6.763 a |
| $CS_1 \times GB_1$ | 24.17 d-g | 0.826 d-g | 6.87 a |
| $CS_1 \times GB_2$ | 21.67 e-h | 0.815 d-g | 6.359 a |
| $CS_1 \times GB_3$ | 17.5 f-h | 0.606 e-i | 6.274 a |
| $CS_2 \times GB_1$ | 21.67 e-h | 0.695 d-i | 6.897 a |
| $CS_2 \times GB_2$ | 15.83 g-i | 0.49 f-j | 7.247 a |
| $CS_2 \times GB_3$ | 12.5 h-j | 0.315 i-k | 7.250 a |
| $K_1 \times GB_1$ | 12.5 h-j | 0.445 g-j | 6.583 a |
| $K_1 \times GB_2$ | 12.5 h-j | 0.378 h-j | 7 a |
| $K_1 \times GB_3$ | 4.167 j | 0.121 k | 2.583 c |
| $K_2 \times GB_1$ | 15.83 g-i | 0.485 f-j | 7.264 a |
| $K_2 \times GB_2$ | 7.5 ij | 0.271 jk | 4.278 b |
| $K_2 \times GB_3$ | 4.167j | 0.095 k | 2.972 c |

$CS_1$, chilling stratification at 5 ºC for 7 days; $CS_2$, chilling stratification at 5 ºC for 14 days; $K_1$, $KNO_3$ 1%; $K_2$, $KNO_3$ 3%; $GB_1$, gibberellin (250 ppm); $GB_2$, gibberellin (350 ppm); $GB_3$, gibberellin (500 ppm); S, (soaking in water for 24 h); C, control; GP, germination percentage; GR, germination rate; MGT, mean of germination time.

Increase of $GA_3$ concentration decreased the germination rate and germination percentage in both species. Gibberellins overcome seed dormancy and bud dormancy in many species, acting as a substitute for low temperatures, long days or red light (Salisbury, 1992). Dormant seeds which require chilling, dry storage after ripening and light as a germination stimulator are often treated with $GA_3$ to overcome their dormancy (Nadjafi *et al*, 2006). $GA_3$ has been exogenously applied as substitute for stratification and has been increased germination in many plant species, including *Leucospermum*, *Fagus sylvatica* and *Heliantus* (Brits *et al.*,1995; Nicolas,1998; Sieler,1998). Earlier reports showed that effect of exogenous applications of $GA_3$ on breaking of seed dormancy and seed germination can be widely differed among and within species (Tigabu *et al.*, 2001). Same to our results, Etemadi *et al* (2010) and Mortensen *et al.* (2004) reported that lower concentrations of $GA_3$ improved germination percentage and germination rate of *Kelussia odoratissima* Mozaff and *Fagus sylvatica*, respectively. They observed that increasing of $GA_3$ concentration decrease germination rate and

germination percentage of these species. Chilling stratification at 5 ° C, for 7 days enhanced germination rate and germination percentage in *S. khuzistanica* and *S. rechingeri* compare to control. Increasing the stratification period to 14 days decreased the germination percentage and germination rate significantly (Table 3). It seems that germination parameters of these species are dependent to period of stratification. Shalaby *et al.* (1997) has been reported that seeds of different *Echinacea* species needed different stratification periods to overcome the dormancy. Also, soaking in water for 24h significantly enhanced germination percentage and germination rate of *S. khuzistanica* and *S. rechingeri* compare to control *(*Table 3.), but other pre-germination treatments have not significantly stimulated their germination. The same results concluded from interaction effect of species and pre-germination treatments *(*Table 4.).

**Table 4.** Interaction effects of seed pre-germination treatments and species on germination percentage and germination rate of *Satureja khuzistanica* and *S. rechingeri.*

| Species | Pre-germination treatments | GP | GR |
|---|---|---|---|
| *S. khuzistanica* | CS1 | 55 b | 1.61 cd |
| | CS2 | 30 e-h | 1.017 d-i |
| | K1 | 23.33 f-k | 0.756 f-l |
| | K2 | 15 i-n | 0.46 i-p |
| | GB1 | 70 a | 2.5 ab |
| | GB2 | 25 f-j | 0.85 e-l |
| | GB3 | 16.67 h-m | 0.52 h-o |
| | S | 46.67 bc | 1.483 c-e |
| | CS1 × GB1 | 15 i-n | 0.54 h-o |
| | CS1 × GB2 | 13.33 i-n | 0.453 j-p |
| | CS1 × GB3 | 13.33 i-n | 0.49 i-p |
| | CS2 × GB1 | 23.33 f-k | 0.81 e-l |
| | CS2 × GB2 | 16.67 h-m | 0.53 h-m |
| | CS2× GB3 | 11.67 j-n | 0.236 m-q |
| | K1 × GB1 | 13.33 i-n | 0.523 h-n |
| | K1 × GB2 | 10 k-n | 0.365 k-q |
| | K1 × GB3 | 8.33 k-o | 0.243 m-q |
| | K2 × GB1 | 15 i-n | 0.493 h-o |
| | K2 × GB2 | 6.66 l-n | 0.213 n-q |
| | K2 × GB3 | 5 l-n | 0.11 o-q |
| | C | 25 f-j | 0.873 e-k |
| *S. rechingeri* | CS1 | 55 b | 1.77 bc |
| | CS2 | 18.33g-m | 0.636 g-n |
| | K1 | 30 e-h | 0.876 e-k |
| | K2 | 20 f-k | 0.673 g-n |
| | GB1 | 75 a | 3.15 a |
| | GB2 | 40 c-e | 1.35 c-f |
| | GB3 | 43.33 cd | 1.793 bc |
| | S | 31.67 d-g | 3.15 a |
| | CS1 × GB1 | 33.33 d-f | 1.113 c-g |
| | CS1 × GB2 | 30 e-h | 1.177 c-g |
| | CS1 × GB3 | 21.67 f-k | 0.723 g-m |
| | CS2 × GB1 | 20 f-l | 0.58 g-m |
| | CS2 × GB2 | 15 i-n | 0.443 j-p |
| | CS2× GB3 | 13.33 i-n | 0.393 k-q |
| | K1 × GB1 | 11.67 j-n | 0.366 k-q |
| | K1 × GB2 | 15 i-n | 0.4 k-p |
| | K1 × GB3 | 0 n | 0 q |
| | K2 × GB1 | 16. 67 h-n | 0.476 i-p |
| | K2 × GB2 | 8.33 k-n | 0.33 l-q |
| | K2 × GB3 | 3.33 mn | 0.08 pq |
| | C | 26.67f-i | 0.97 d-j |

$CS_1$, chilling stratification at 5 ºC for 7 days; $CS_2$, chilling stratification at 5 ºC for 14 days; $K_1$, $KNO_3$ 1%; $K_2$, $KNO_3$ 3%; $GB_1$, gibberellin (250 ppm); $GB_2$, gibberellin (350 ppm); $GB_3$, gibberellin (500 ppm); S, (soaking in water for 24 h); C, control; GP, germination percentage; GR, germination rate; MGT, mean of germination time.

The highest germination percentage and germination rate were observed in both species at low concentration of $GA_3$ (250ppm), followed by chilling stratification at 5°C, for 7 days. It can be concluded that the studied species have physiological dormancy. Physiological dormancy is regulated in seeds due to relative levels of growth promoters ($GA_3$) and inhibitors as Abscisic acid (ABA). For increasing of germination in seeds, $GA_3$ concentration should exceed the level of ABA. Stratification by cold water or direct use of $GA_3$ changes the endogenous levels of these phytohormones thereby breaks the seed dormancy (Gupta, 2003). Therefore use of low concentration of $GA_3$ (250 ppm) or chilling stratification stimulate seed germination in these species and showed a larger effect than the other treatments applied in this study.

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