RESEARCH ARTICLE

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Optimal treatment increased the seed germination of *Salvia verticillata* L.

ABSTRACT

Most seeds of the medicinal species are variable regarding their ecological compatibility with environmental conditions. Therefore, identifying the ecophysiological factors that affect dormancy and create optimal conditions for seed germination of medicinal plants is necessary for their culture and production. To evaluate the effect of different treatments on seed germination of medicinal species of *Salvia verticillata*, collected in the summer of 2010 in Eastern Azarbaijan, we have performed completely randomized experimental tests with 4 replications. The experimental design of treatment prior to growth included: scrape the skin with sandpaper, treatment with 500 ppm gibberellic acid for 24 and 48 h, treatment with citric acid for 10, 20 and 30 minutes, chilling for 2 and 4 weeks, treatment with warm water at 70°C and control treatment. Results showed that the effect of different treatments was significant on seed germination percent of the medicinal plant *Salvia verticillata*. Scrape the skin with sandpaper, citric acid treatment for 10, 20 and 30 minutes, and gibberellic acid treatment for 24 hours, increased the germination percentage compared to the control treatment. The most positive impact was observed on the dormancy breaking and germination of medicinal species *Salvia verticillata*.

Key words: *Salvia verticillata*, seed dormancy, germination, treatment prior to growth

Introduction

Plant seeds are very important from the perspective of the biotechnology and plant industry. They can be used as food, as fodder for livestock and for production of medical products, cosmetics and other industrial materials. On the other hand, since the seeds are genetic heritage of previous plants, they could be used for different research purposes and for selection of plants with better characteristics (El-Bramawy & El-Sarag, 2012; Bello & Igbokwe, 2013). Seed dormancy is actually a physiological phenomenon faced by many crops or wild plants (Tajbakhsh & Ghiyasi, 2008). Dormancy and seed germination are dependent from the genetic factors and environmental conditions influencing the growth and development of the plants, as well as the situation after the harvest (Sarmadnia, 1996). Factors that affect the seed dormancy include: seed shell (shells impermeability to water, oxygen and mechanical resistance), embryos (embryos being depressed, immature embryos) and presence of inhibiting substances in the seeds. There are several ways to stimulate germination of dormant seeds (Latifi, 2001; Elamin et. al., 2013). In case of lack of minimal environmental conditions, the seeds can stay inactive keeping their proliferative capabilities (Khosravi, 1996). The ability of seeds to delay germination until establishment of suitable location and time is one of the most important mechanisms for their survival (Copeland & McDonald, 2008). Having one type of dormancy, the seeds of many medicinal and grassland plants in natural habitats ensure their survival for many years, but for propagation and cultivation of these plants it is essential to release the seed for germination (Tajbakhsh, 1996; Javanmiri Pour et. al., 2013). Considering the importance of medicinal plants for treatment of different disease, and also limited natural habitats and slightly breeding, planning of their cultivation and domestication is necessary (Soltanpoor et al., 2009).

Purple salvia is a perennial plant of the genus *Salvia* that belongs to the family Labiatea (Ghahraman, 1984), which has

RESEARCH ARTICLE

medicinal properties (Delnavaz Hashemian & Ataii Azimii, 2006; Souri et al., 2005). This plant grows as a wild form in various parts of Iran such as Tehran, Azerbaijan, Qazvin and Mazandaran (Souri et al., 2005).

Due to low seed germination of *Salvia verticillata* L. and its extreme importance for the herbal medicine, it is of crucial imporance to examine the factors that affect the germination and dormancy of this plant. Today, the use of some combinations of pretreatment has been suggested in order to stimulate seed germination, reduce the time between planting and seed germination, and forcing the synchronicity in seed germination with possibility of germination in other environmental conditions (Bahadori & Javanbakht, 2006). Hejabi & Soltanipoor (2006) showed that pre-eruptive mechanical scarification can influence the germination characteristics of *Salvia mirzayanii* increasing the seed germination of this medicinal plant. Shakeri-Almoshiri et.al. (2009) also concluded that seed germination of *Teucrium polium* is increased substantially after treatment with sandpaper, compared to non-treated seeds.

In order to stimulate germination of *T. polium*, Kochacki & Azizi (2005) observed that treatment with 500 ppm gibberellic acid had the most positive impact on seed germination. More studies have been done with hot water treatment showing that this type of treatment had an effect on the seed germination of many medicinal plants and rangeland. It has been showed that warm water treatment of *S. mirzayanii* significantly influenced the seed germination (Hejabi & Soltanipoor, 2006). Treatment with cooling at 5°C for 2 weeks increased germination of *Ferula gomussa* (Macchai et.al., 2001).

Salvia is one of the largest genera within the family *Labiateae*, with nearly 1000 species in the world. Almost all *Salvia* species are used for medical applications. Essential oils of some *Salvia* species are used in the pharmaceuticals, perfumery, health products and cosmetics, as flavors and seasoning in the food industry (Zargari, 1990). Thus, providing information on the quality characteristics of the seeds and creating optimal conditions for seed germination is necessary for cultivation and their reproduction (Ghasemi Pirbaloti et al., 2007).

Due to the medicinal importance and problematic seed germination of these plants, the examination of factors affecting the germination and propagation of the seeds is of great imoprtance.

Materials and Methods

Plant materials

The seeds of *Salvia verticillata* L. were collected during the summer of 2010 from rangelands of Kalybar, northwest of the East Azerbaijan with longitude 47°2', latitude 37°52', and a height of 1383 meters. This is located within the forests Ghale daresee, on the paths towards area of protected forests of Arasbaran (Shadkami-Til, 2013a; 2013b; 2013c).

Preparation of samples

After collecting 100 seeds from a plant, the samples for each treatment were randomly selected. The experimental design was completely randomized with 4 replicates and was used for each replication of 25 seeds. In order to disinfect the seeds, they were placed for 3 minutes in a solution of 75 cc of distilled water and 25 cc of sodium hypochlorite. Then, the seeds were removed from the solution and washed well with distilled water until disappearance of all traces of the solution. In the next step, sterile filter paper was added as planting bed to the cultivating containers.

Seed treatment

Scrape with sandpaper

In order to perform scrape treatment, the seed hulls by sandpaper (between two layers of sandpaper) and pulverized until thinning of their shells. After performing the scrape treatment, the seeds were placed in water for 24 hours. After this period of time, treated seeds were transferred in a cultivation container containing wet filter paper and were kept at temperature of 25± 3°C (Aliero, 2004; Hajebi & Soltanipoor, 2006; Makkizadeh et al., 2006; Sakeri Almoshiri et al., 2009; Tavili et al., 2009; Uzun & Aydin, 2004).

Gibberellic acid treatment

For gibberellic acid treatments, the seeds were soaked in water for 48 hours. Then the seeds were put in a container, and 500 ppm gibberellic acid solution was added. In these conditions they were kept for 24 hours (100 seeds) and 48 hours (100 seeds). After this treatment, the seeds were washed with water and then transferred to a container culture (Amooaghaie, 2007; Bahadori & Javanbakht, 2006; Ghasemi Pirbaloti et al., 2007; Khochaki & Azizi, 2005; Makkizadeh et al., 2006).

Chilling

In the chilling treatment, the seeds were soaked in water for 48 hours and then were maintained for 2 weeks (100

RESEARCH ARTICLE

seeds) and 4 weeks (100 seeds) in moist sand in sterile plastic bags at 4°C. The seeds were kept steadily moist in cold. After the specified time, the seeds were placed to germinate at room temperature (Durrani et al., 1997; Jankju Borzelabad & Tavakkoli, 2008; Macchai et al., 2001; Makkizadeh et al., 2006; Soltanpoor et al., 2009).

Warm water

Seeds (100 seeds) were poured in the container and added 70°C water. Then were left at room temperature for 24 hours and allowed to cool. After 24 hours the seeds were transferred to a container culture (Hajebi & Soltanipoor, 2006; Makkizadeh et al., 2006; Patane & Gresta, 2006; Soltanpoor et al ., 2009; Tavili et al., 2009).

Citric acid

Seeds were separately poured in a container and to each was added citric acid. After 10 min (100 seeds), 20 min (100 seeds) and 30 minutes (100 seeds), they were removed and washed with abundant water to stop the effect of the citric acid. Then the seeds were placed for 24 hours in a container of water, and after 24 hours were transferred to a container culture (Jones, 1963; Khochaki & Azizi, 2005).

Control treatment

The seeds were placed in a container culture and irrigating daily with distilled water to maintain the moisture (Ghasemi Pirbaloti et al, 2007).

Cultivation

After treatment, the seeds were cultivated within the planting bed and placed on filter paper. Germination counting was started from the third day of planting and was performed daily for 21 days. While supplying adequate moisture, germination changes were recorded according to the special forms of viability. Results were observed daily and the number of seeds in each treatment was recorded. All germinated seeds were counted and removed from the container. After performing the all steps within the prescribed time, germinated seeds and germination percentage were calculated by using the following formula according to the method Panwar (Panwar & Hardwaj, 2005):

Germination rate = n/N*100 (%)

where n= total number of germinated seeds during the period, N= total number of planted seeds.

Data analysis

To investigate normality of data, we used Kolmogorov Smirnov test. In order to compare overall, we conduct the analysis of variance and Duncan test to compare the means. Data analysis was performed using SPSS software version 14 (Bihamta & Zare Chahoki, 2009).

Results

Scrape with sandpaper

The comparison of germination percentage between the scarification and control treatment showed that there is a significant difference at level of 5% (Table 1). Scarification with sandpaper treatment showed a higher percentage of germination compared to the control treatment (Figure 1). Germination percentage of seeds scratched with sandpaper was 53%, where in the control treatment this value was 33%. After scarification treatment the seed germination increased by 20%.

Table 1. *Analysis of variance to compare percentages of germination between scarification and control treatment*

Treatment	Mean	Standard deviation	Standard error	t	P
Scarification	53	6.83	3.41	3.735	0.010*
Control treatment	33	8.24	4.12		

Significant differences at 5% level

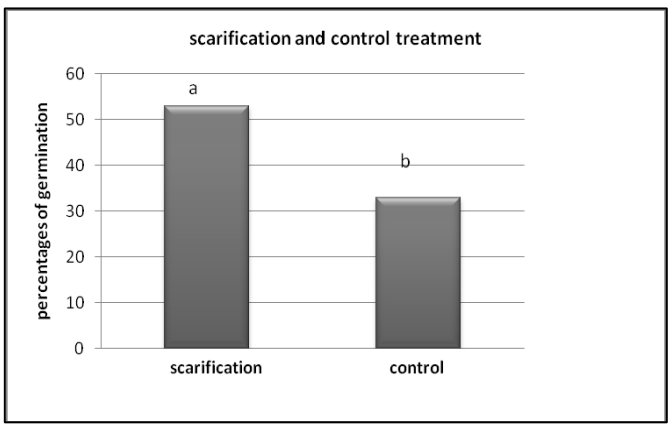

Figure 1. *Compared percentages of germination between scarification and control treatment.*

Gibberellic acid treatments for 24 and 48 hours

Comparison of percentages of germination between the treatments with 500 ppm gibberellic acid for 24 and 48 hours, and the control treatment showed a significant difference at 1% (Table 2). Treatment with 500 ppm gibberellic acid for 24

RESEARCH ARTICLE

hours represents the highest germination percentage with a numerical value of 50% (Figure 2). The lowest germination percentage related to 500 ppm gibberellic acid treatment was the treatment for 48 hours with a numeric value of 28%. There is not a significant difference between the control treatment and treatment with gibberellic acid for 48 hours (Figure 2). The results showed that with increasing of the time of treatment with gibberellic acid, germination percentage of the seeds decreases.

Table 2. *Analysis of variance to compare germination percentage of gibberellic acid and control treatment*

Source	DF	Mean-square	Sum squares	F	P
Between groups	2	532	1064	16.86	0.001**
Within groups	9	31.56	284		
Total	11		1348		

Significant differences at 1% level

Citric acid treatments for 10, 20 and 30 minutes

Comparison of germination percentage between the treatments with citric acid for 10, 20 and 30 min and the control treatment showed that there is a significant difference at a level of 5% between all treatments (Table 3). Figure 3 shows that the highest percentage of germination was obtained in seeds treated with citric acid for 10 minutes with a numerical value of 50%. Compared with the control treatment it was increased with 33%. The lowest percentage of seed germination was observed in the control treatment with a numerical value of 33%. Germination percentages in the treatments with citric acid for 20 minutes and 30 minutes were 40% and 38%, respectively, which were also increased comparing to the control treatment. Results showed that in the citric acid treatment with time period of 10, 20 and 30 min, germination percentage significantly increased compared to the control treatment (Figure 3). With increasing the duration of treatment, there was a difference in the reduction in the rate of germination. There was not a significant difference between the control group and the treatment with citric acid for 30 minutes.

Chilling and hot water treatments

Comparison of the percentages of germination between the treatment with 70°C warm water, chilling 2 and 4 weeks and the control treatment showed a significant difference at a level of 5% (Table 4).

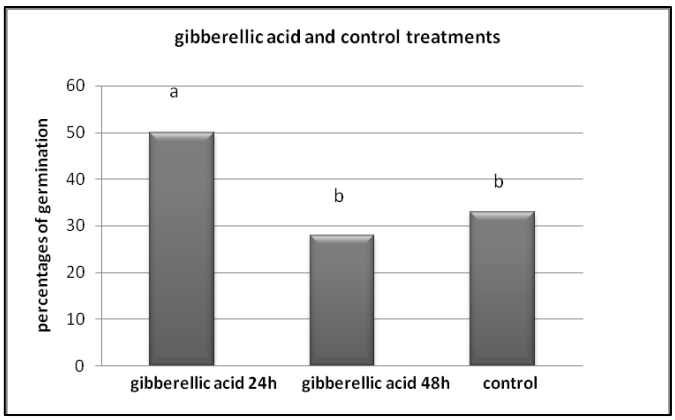

Figure 2. *Compared percentages of germination between gibberellic acid and control treatments.*

Table 3. *Analysis of variance to compare percentages of germination between citric acid and control treatments*

Source	DF	Mean-square	Sum squares	F	P
Between groups	3	203.67	611	3.94	0.036*
Within groups	12	51.67	620		
Total	15		1231		

Significant differences at 5% level

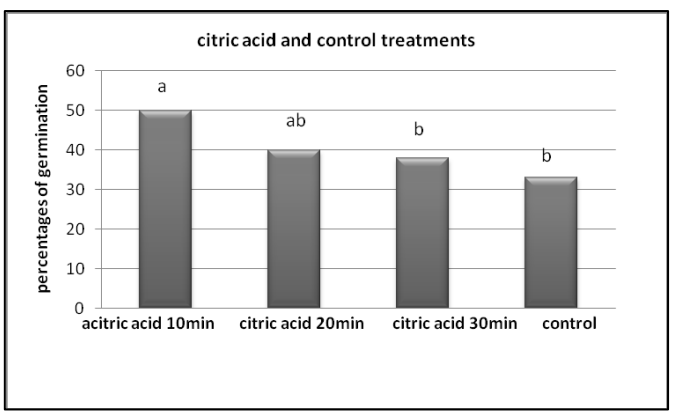

Figure 3. *Compared percentages of germination between citric acid and control treatments.*

According to the comparison indicators (Figure 4) control treatment showed the highest percentage of germination (33%) among treatments. Germination rates reduced to 13% after treatment with 70°C warm water. A numeric value of 16% seed germination was observed for the 2 weeks chilling treatment. The minimum germination percentage was counted for the chilling treatment of 4 weeks (8%).

RESEARCH ARTICLE

This germination rate decreased with 25% compared to the control treatment. There was not a significant difference between the treatments chilling 4 weeks and chilling 2 weeks (Figure 4).

Table 4. *Analysis of variance to compare percentages of germination between treatments of warm water, chilling and control treatment*

Source	DF	Mean-square	Sum squares	F	P
Between groups	3	434.67	1304	5.134	0.016*
Within groups	12	84.67	1016		
Total	15		2320		

Significant differences at 5% level

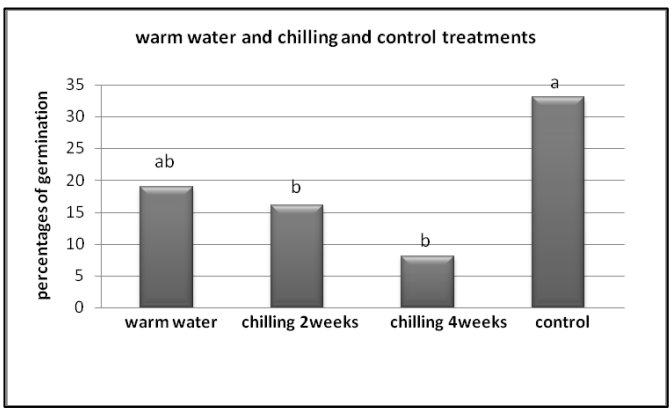

Figure 4. *Compared percentages of germination between treatments of warm water, chilling and control treatment.*

Comparison of results between all treatments (scarification with sandpaper; 24 and 48 h with gibberellic acid; 10, 20 and 30 min with citric acid; 2 and 4 weeks chilling; warm water 70°C)

Results from the analysis variance indicated significant differences among all treatments for germination percentage at 1% (Table 5). Comparison of germination percentage in all treatments showed that the highest germination percentage with a numeric value of 53% was observed in the scarification with sandpaper, which compared to the control treatment was increased by 20% (Figure 5). Next higher percentages of germination were obtained for the citric acid treatment (10 min) with a numerical value of 50% and gibberellic acid treatment for 24 hours with the same value. Germination rates in these treatments have increased by 17% compared to the control (Figure 5). In the treatment with citric acid for 20 min the germination percentage was 40%, and for the citric acid treatment for 30 min this value was 38%. The increase was 7% and 5%, respectively compared to the control treatment. In the treatments with gibberellic acid for 48 hours (28%), warm water (20%) and a chilling for 2 weeks (16%), we observed reduction in germination respectively with 5%, 13% and 17 %. Results showed that the minimum percentage of germination was for the chilling 4 weeks.

Table 5. *Analysis of variance comparing the percentage of germination in all treatments*

Source	DF	Mean-square	Sum squares	F	P
Between groups	9	970.89	8738	17.63	0.0**
Within groups	30	55.07	1652		
Total	39		10390		

Significant differences at 1% level

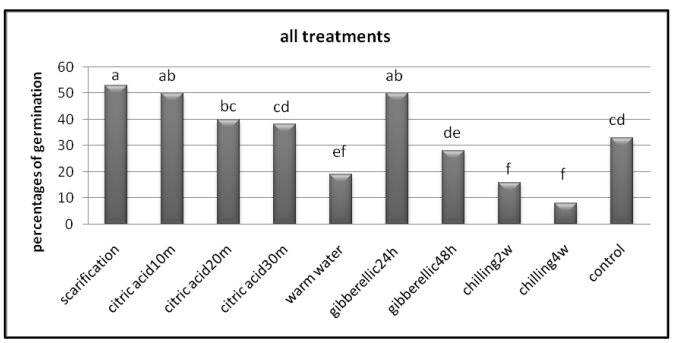

Figure 5. *Compared germination percentage in all treatments.*

Discussion

Treatments performed in this study were selected according to the type of species and ecological conditions. A significant increase and highest seed germination of *Salvia verticillata* L. we observed for the scarification with sandpaper. Shakeri-Almoshiri et al. (2009) showed the positive impact of the sandpaper for stimulation of the seed germination of *Teucrium polium* L. Scarification with sandpaper caused thinning seed hull and thereby reduced the mechanical strength out of the bud. Being successful seed germination of *S. verticillata* L. under the scarification with sandpaper, confirms the impact of mechanical strength hull against out of bud. In this study, the germination percentage of seeds treated with gibberellic acid for 24 h significantly increased compared to the control treatment.

RESEARCH ARTICLE

Our results are in agreement with the findings of Fulbright et al. (1983), which stated that the gibberellic acid causes stimulation of the seed germination. The physiological dormancy is controlled by chilling and gibberellic acid (Fulbright et al, 1983). Growth regulating substances have a key role in the creation and control of seed physiological dormancy. Among these substances is the gibberellic acid that controls the seed dormancy by stimulation of germination. The role of gibberellic acid has been confirmed in overcoming the dormancy of seed hull. Gibberellic acid is able to stimulate the germination increasing all germination characteristics (Nadjafi et al., 2006).

In this study, the most seed germination of *Salvia verticillata* L. was observed at 500 ppm gibberellic acid for 24 hours. Increasing the duration of treatment from 24 to 48 hours can be effective in reducing germination indices. Gibberellic acid treatment for 48 hours resulted in reduction of germination indices. Results of this research corresponded to findings of Tavili et al.(2009), that 500 ppm gibberellic acid reduces germination rates of *Gundelia tournefortii*. With increase the duration of exposure with gibberellic acid of the seeds of *Salvia verticillata* L., the negative effects on the dormancy breaking are also increased. Probably this acid eliminates the plant embryo with the time.

Physiological studies have shown the effect of chilling on the seeds and their germination due to changes of the hormones within the seed and increase of gibberellin concentration. After activation of degrading enzymes in stored seeds, these hormones cause provision of food and feeding the embryo, and ultimately seed germination. Most specialists believe that these hormones can be replaced by removing the need for chilling seed or even beyond, all factors that affect the seed germination (Bewly & Blak, 1994; Nasiri, 1984). During cold periods, the seed is influenced by a set of internal and external processes, which result them over time and eventually will lead to seed germination. Only some of those processes can reduce disincentives and increase stimulants to stimulate seed germination. In other words, a result applied chilling of the seed and creation the proper hormonal balance leads to stimulation of seed germination and it is not influenced absolutely by a particular hormone. So, seeds that have been exposed to chilling for sufficient time, contents of the internal hormones were adequate for germination and it is not need to use external stimulation (Nasiri, 1984; 1985; Sarmadnia, 1996). Another important effect of chilling, especially when at the same time is freezing and thawing, is reduced the mechanical strength of the seed hull (Bewly & Blak, 1994; Nasiri et al., 2003).

The best treatment for breaking is chilling. Results of this study showed that the chilling treatment for 2 and 4 weeks significantly reduced the germination percentage compared to the control treatment. Reduced germination at low temperatures could be due to the negative effect of the low temperatures on the enzyme activity, and therefore reduce metabolic and biosynthetic activity required for theb seed germination, growth and development. Results showed that placing the seeds in warm water (70°C) caused a significant decrease in the seed germination index of *Salvia verticillata* L.. Warm water can reduce the resistance hull of the seed against out the seedlings through changing the permeability hull. While immersion of the seeds in warm water is proper for permeabilization of the hull, but it significantly reduced seed germination. The reason for this could be due to the penetration of warm water into the structure of the seed and the adverse impact on the seed tissues (Tavili et al., 2009).

Citric acid is capable to create the crack in hull of the plant and greatly to reduce the role of hull in inhibition of the germination process. Citric acid treatment for 10 min increased all germination indicators compared to the control treatment. Treatment for 20 and 30 min resulted in increased germination percentage and germination ability compared to the control treatment. Citric acid is capable to reduce the strength of seed hull and its role in the inhibition causing increased germination and optimization of this process. Highest indexes were achieved in the treatment with citric acid for 10 minutes. A decreasing trend was observed with increasing the time of treatment with citric acid, so that the rise of time caused decreased germination indices. This could be possibly due to the adverse impact of the acid on the seed tissue. Our results corresponded with the findings of Nasiri et al. (2003) that citric acid increased germination of *Eremurus olgae* Regel. According to the obtained results the highest percentage of germination was observed in treatment scratch hull with sandpaper. Chemical treatments were also effective for the seed germination of *Salvia verticillata* L.

Since the gibberellic acid usually affects the breaking of dormancy caused by metabolic factors, it can be suggested that probably seed dormancy of *Salvia verticillata* L. is mainly due to mechanical constraints of hull and somewhat affected by the metabolic constraints.

RESEARCH ARTICLE

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RESEARCH ARTICLE

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