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Effects of treatments of gibberellic acid, citric acid and stratification on germination of seeds of *Salvia siirtica* Kahraman, Celep & Doğan sp. nov. (Lamiaceae)

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Abstract

Salvia siirtica Kahraman, Celep & Doğan sp. nov. (Lamiaceae) have been recently reported as a new species from southeaster Turkey. It is currently grown in remote areas, where locally adapted and sparsely populated. The study will be of paramount importance for understanding of germination behavior in *Salvia siirtica*, possibly due to encouraging its propagation, but not being endangered. For this reason, conducted a study, consisting of a set of germination tests, under the tissue culture laboratory. In this study, *Salvia siirtica* seeds were treated with gibberellic acid (24 h and 48 h in GA3 of 250 mg l-1), citric acid (5 and 10 min in its solution of 4 %) and stratification (14 and 28 day in fine sand maintaining in refrigerator of + 4 °C). Experiment was set up in completely randomized factorial design with three replications. Comparing with control, sole gibberellic acid treatment or combined with other treatments had no effect on *Salvia siirtica* germination and rooting behavior. But, combined effects of citric acid and stratification treatments were statistically significant ($p<0.01$). The highest germination and rooting rates (21.25 and 17.97 %, respectively) were measured on stratification treatment of 28 day.

Keywords: *Salvia siirtica*, seed germination, gibberellic acid, citric acid, stratification

INTRODUCTION

Salvia L. is the largest genus of the Lamiaceae family, exhibiting a wide distribution with approximately 1000 species. With three different gene centers, namely Central and South America (500 spp.), West Asia (200 spp.), and East Asia (100 spp.), it is widely found in the tropical and temperate regions of the old and new world (1). With a total of 97 species reported in Turkey, Turkey is an important gene center for Salvia in Asia [2].

The sage species are important plants whose medical characteristics have been known since ancient times and were named as “elelisfakon” and “sfakon” by Hippocrates, Theophrastus and Dioscorides. They were known by Ancient Egypt and the Chinese as plants that improved brain functions. In the 12th century, Sinte Hillgarde defined sage as a true antidote, a panacea plant. In the 16th century, physicians of the Salerne School had statements expressing the benefits of sage as “if you have planted sage in the garden, there is no need to die”. The Salvia L. species, also called sage, have been used in the treatment of more than sixty diseases since ancient times. The most common of these include pain relievers, treatment of epilepsy, colds, bronchitis, tuberculosis, hemorrhages and menstrual disorders [3, 4]. While Sage belongs to the group of medicinal and aromatic plants and used in treatments, there is also information indicating that some species of this genus have also been used as ornamental plants [5]. The sage plant is shown as an ornamental plant, which likes sun or semi-shade environments [7] and used in rock gardens [6]. Salvia officinalis shown in the category of some medicinal and aromatic plant classes that can be used as ornamental plants [8] suggests the possibility of classifying other Salvia species as ornamental plants and their use in landscape design. Similarly, it is said that Salvia species are annual and perennial plants with a best blooming in summer, and details of their use as ornamental plant in landscaping has been provided through some sage species (*S. coccinea, S. dorisiana, S. elegans, S. farinacea, S. patens, S. splendens, S. viridis, S. discolor, S. officinalis*, etc.).

While seed germination has a significant effect on the success of the plant breeding cycle according to Bu et al., 2008, the differences in germination percentage according to Grime et al., 1981 and Nishitani and Masuzawa, 1996 are generally seen as the effect of specific ecological conditions [9].

According to Lorite et al., 2007, seed germination studies on rare and endemic species play an important role in the identification of species conservation strategies for these plants. According to Fenner and Thompson, 2005, seed germination is the only way to protect the genetic diversity of populations [8]. According to Blanca et al., 1998, Moreno-Saiz et al., 2003, these species are also at risk of extinction in many cases, and therefore, the accurate and precise understanding of the germination abilities of these taxa is important for their protection and the continuity of the species [9].

Salvia siirtica Kahraman, Celep and Doğan sp. (Lamiaceae) is described and shown as a new species that grows in oak forest openings in the north of Siirt province in Southeast Anatolia. *Salvia siirtica* is known only from its type locality, and the number of individuals and geographical range seem very small. The estimated distribution area is less than 10 km2 and the number of mature individuals is less than 1200. *Salvia siirtica*, which has a very low number of individuals and is stuck in such a narrow area of proliferation, is facing the danger of extinction. The most important factor threatening the existence of this species in the region is human beings. Activities such as overgrazing and dam construction threaten the existence of the species. The last place that the species is able to survive and find a habitat is the village cemetery. For this reason, *S. siirtica* is among the ‘Critically endangered’ species category (CR) (IUCN 2001) (2).

For this reason, it is extremely important to determine

the optimum germination conditions of the plant and then to reproduce it in its natural conditions. Thus, it has become necessary to carry out such a study in order to determine the germination conditions of the plant.

MATERIALS and METHODS

Sage seeds were collected after maturation from Tillo district of Siirt province (in oak forest openings at 37 degrees 58 minutes 13 seconds north - 42 degrees 01 minutes 53 seconds east, 1473 m altitude) in November.

Figure 1. Distribution of *Salvia siirtica* [10]

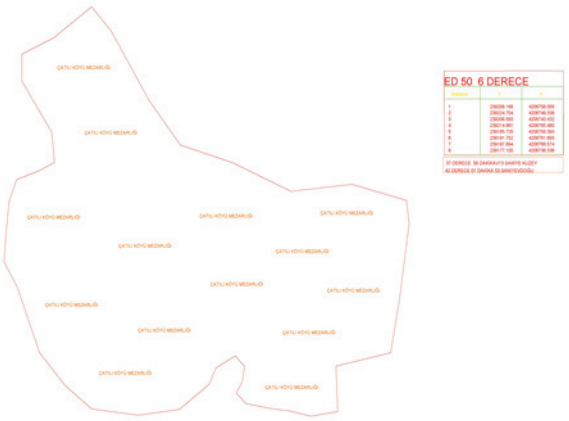

Figure 2. Siirt Province Tillo district Çatılı village cemetery, the only area where *Salvia siirtica* is located, and *Salvia siirtica* distribution in the cemetery

Sterilization of collected seeds was carried out in a sterile cabinet for 5 minutes with 25% NaOCl (commercial bleach-Domestos). The seeds, which were rinsed 3 times with sterile distilled water, were kept in sterile water for 48 hours. After 48 hours, four different methods were applied to the seeds for germination.

Control: Seeds were planted on sterilized drying paper in glass petri dishes without any treatment.

Gibberellic acid (GA3) treatment: Seeds were cultured in glass petri dishes after being kept in GA3 at a concentration of 250 mg l-1 for 12, 24, 36 and 48 hours.

Hot water treatment: The seeds were placed in sterile water heated to 70°C and allowed to cool in room conditions for 24 hours before being cultured on glass petri dishes.

Citric acid treatment: The seeds were placed in a citric acid solution prepared at a concentration of 4% for 5 and 10 minutes, then rinsed with sterile distilled water, and kept in sterile distilled water for 24 hours. The seeds were then planted in glass petri dishes.

Cold treatment: Seeds were planted in sterile glass petri dishes inside moist sand sterilized in autoclave. Petri dishes were stored at + 4°C in the refrigerator for 1, 2, 3 and 4 weeks. At the end of the storage period, the seeds were rinsed with sterile water and cultured as in other treatments.

The study was conducted in Siirt University Faculty of Agriculture, Department of Field Crops Tissue Culture Laboratory under sterile conditions with 3 replications according to "Randomized Lots Test Pattern". In each recurrence, the seeds were planted in glass petri dishes, 20 seeds each, on coarse filter paper, and watered with sterile distilled water as needed. All steps of the treatments were carried out under sterile conditions and the petri dishes with the seeds were kept in darkness in a climate chamber set at 18±2 °C. Daily observations were made and recorded in order to calculate rooting and germination percentage ratios of the seeds. The obtained data were analyzed by JUMP statistical software and the average % rooting and germination values were calculated.

RESULTS and DISCUSSION

Among the treatments, no germination was observed in Control and Gibberellic acid (GA3) treatments. For this reason, no statistical analysis was performed for these treatments.

Table 1. Effects of +4 C cold folding and Citric acid treatments on germination and spreading

Treatments	Germination (%)	Spreading (%)
Stratification (14 in fine sand maintaining in refrigerator of + 4 °C)	5,26 bc	5,263 bc
Stratification (28 day in fine sand maintaining in refrigerator of + 4 °C)	21,25 a	17,92 a
Citric acid (5 in its solution of 4 %)	2,63 c	2,63 c
Citric acid (10 min in its solution of 4 %)	9,80 b	7,84 b
Cv	31,92**	24,41**
LSD	6,21	4,10

**: Significant at %1

When Table 1 is examined, it can be seen that the difference between citric acid and cold folding treatments is statistically significant at 1% level. The highest germination rate was obtained from cold folding treatment for four weeks at + 4°C with 21.25%, while the highest rooting rate was obtained from the same treatment with 17.97%. The lowest germination rate was obtained with citric acid treatment for five minutes (2.63%), while the lowest rooting rate was also obtained with citric acid treatment for five minutes (2.63%).

Many germination studies have been carried out on sage in laboratory conditions. In studies conducted on S. aegyptiaca species, the effects of temperature and salinity on the germination biology of seeds were investigated (11). According to the results of this study, temperatures between 10-40°C were determined as the desired temperature. Germination rate in seeds exposed to salt stress is reduced or non-existent. In a study on Salvia dicroantha species, it was found that the seed weight significantly affected the germination rate (12). The effects of light/dark, GA3, and temperature treatments have been determined; and the GA3 treatment was found to be ineffective. The highest germination rates were found to be 45% in 16/8 hour photoperiod, and 49.44% in 20 °C

treatment. Germination biology was examined by applying different treatments of GA3 and temperature to the seeds of the endemic species Salvia smyrnaea sage [9]. Some of the seeds were subjected to folding at +5 °C for 45 days before planting. At the end of the study, it was found that unfolded seeds had a germination rate between 6.6-50%, whereas folded seeds had a germination rate of 13.3-56.6%. For the germination test on Salvia officinalis seeds at different temperatures, the seeds were kept in water for various periods before planting [13]. At the end of the study, it was found that seeds kept in water for 12 hours at 30 °C had the highest germination rate. In another study where seeds of Salvia verticillata species were treated with sanding and hot water, it was found that the effect of sanding on germination was 53%; and that of hot water treatment was 19% [14]. In another study conducted by the same researches on sage species, different methods were used this time (15). Seeds were kept at 500 ppm GA3 for 24 and 48 hours; 10, 20 and 30 minutes in citric acid; folded for 2 and 4 weeks; kept in 70 °C hot water, and sanding was also applied to the seeds as a different treatment. At the end of the study, the lowest germination rate was obtained from 4 weeks of folding, and the highest germination rate was obtained from sanding.

In this study conducted on *S. siirtica* species, the highest germination success among treatments was obtained from 4 weeks, +4 °C cold folding. When previous studies are taken into account, it should be considered that the first point to be noted is the plant species being studied. Because even though they are members of the Salvia genus, there can be a difference between the species' germination wishes, as well as any similarities. The high temperature of the Siirt region ecology may have influenced the germination biology of the seeds. Considering the temperature regimes applied in other studies, it should be taken into account that the controlled condition in this study is provided at only 18±2 °C. As it can be seen, GA3 treatment did not encourage germination in every study done, and if there was any effect at all, it was found to be effective at different rates. The treatment temperature is also important as well as the effect of GA3 concentration applied. In a germination study it is stated that the same concentration of GA3 stimulates germination differently at different temperatures [16]. Another view that supports this statement and out study is that; GA3 does not behave as a standard and may vary depending on plant species and germination conditions [17]. Cold folding is a treatment that provides positive results in our study. Regarding this, it can be stated that the cooling of the seeds for a sufficient time causes the content of the internal hormones to become sufficient for germination and there will be no need for external stimuli [17, 18]. It should be noted that the highest germination and rooting rate in this study is obtained after cold treatment. In this study, where citric acid treatment is also effective, it can be said that success is achieved by abrading the shells of the seeds. It is proposed that citric acid reduces the strength of the seed coat and its role in its inhibition, leading to an increase in germination [15]. In this study, a 10-minute wait was more effective than a 5-minute wait.

CONCLUSION

If we consider the utmost benefit we can obtain from a plant, increasing the usage areas of plants that are cultivated and cultured, produced and reproduced, will also allow us human beings to meet our psychological, physical, medical, and other needs. The first requirement of using a plant is to produce it, and the first requirement of production is to eliminate the conditions that prevent production. The first and most fundamental step to initially protect and increase the number of individuals of *S. siirtica*, the topic of our research, within its limited habitat, ensuring its sustainability and continuation of the species, and afterwards determine areas of use according to its characteristics is to ensure germination. In this study, conducted with this purpose, it was determined that seeds showed germination and rooting in citric acid and cold treatments. Other treatments did not result in success. This experiment, which was conducted to obtain more successful germination and rooting from seeds of *S. siirtica*, can be regarded as a preliminary study. In a following study, seeds are considered to be cultivated at different concentrations and durations with these two successful treatments, either individually or in combination. With the seedlings obtained as a result of the increase in germination and rooting, it is possible to protect, and ensure the survival of *S. siirtica* species, which has so far been known to have a limited presence and a narrow distribution. With the increasing number of individuals, it can be possible to reach the data we expect to obtain, and awareness of their usage can be established.

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REFERENCES

[1] Walker, J. B. and Sytsma, K. J. 2007. Staminal evolution in the genus Salvia (Lamiaceae): molecular phylogenetic evidence for multiple origins of the staminal lever. _ Ann. Bot. 100:375_391.

[2] Kahraman A. Doğan M. and Celep F. 2011. *Salvia siirtica* sp. nov. (Lamiaceae) from Turkey, Nordic Journal of Botany 29: 397_401, 2011

[3] Rivera D. Obon C. Cano F. 1994. The botany, history and traditional uses of three-lobed sage (Salvia fruticosa Mill.) (Labiateae). Economic Botany, 48: 190-195.

[4] Topçu G. 200). Bioactive triterpenoids from Salvia L. species. Journal of Natural Products, 69: 482–487.

[5] Seçmen, Ö. Gemici, Y. Görk, G. Bekat, L. and Leblebici E. 2000. Flowering Plants Systematics. Ege University Science Faculty Books Series No: 116. İzmir.

[6] https://samsun.tarim.gov.tr/Belgeler/Yayinlar/ Kitaplarimiz/sus_bitkileri_yetistiriciligi.pdf (Access:06.08.2017)

[7] http://megep.meb.gov.tr/mte_program_modul/ moduller/S%C3%BCs%20Bitkileri.pdf (Access: 06.08.2017)

[8] http://bahcebitkileri.cu.edu.tr/upload/ymendi/ susbitkileri.pdf (Access: 06.08.2017)

[9] Subaşı Ü. Güvensen A. 2010. Seed germination studies on rare endemic Salvia smyrnaea Boiss. (Lamiaceae), Biological Diversity and Conservation 3/3 (2010) 126-132

[10] https://earth.google.com/web/@37.07257493,41.30231231,-406.60796044a,2794863.29368025d,35y,0.00001263h,1.80463047t,0.00000085r/data=CkcaRRI_CiQweDQwMGM4N2E4ZTJmNTg3NGI6MHhiMGQ4ZWZmMDk4MjcyMjIZ_BadLLX2QkAhl8YvvJL4REAqBVNpaXJ0GAIgAQ (Access: 06.08.2017)

[11] Gorai, M. Gasmi, H. Neffati, M. 2011. Factors influencing seed germination of medicinal plant Salvia ae-

gyptiaca L. (Lamiaceae). Saudi J Biol Sci. 2011 Jul; 18(3): 255–260.

[12] Demir S. Özdener E. ,İncedere Y. D. 2007. Effect of temperature, light, seed weight and GA3 on the germination of Verbascum bithynicum, Verbascum wiedemannianum and Salvia dicroantha. Pak J Biol Sci. 2007 Apr 1;10(7):1118-21.

[13] Dastanpoor N. Fahimi H. Shariati M. Davazdahemami S. Hashemi S.M.M. 2013. Effects of hydropriming on seed germination and seedling growth in sage (Salvia officinalis L.). African Journal of Biotechnology, 12(11): 1223-1228.

[14] Khakpor A. Bibalani G.H. Mahdavi K. 2011. Influence of scabbing and hot water treatment on germination of Sage plant (Salvia verticillata) seeds. Annals of Biological Research, 2 (5): 52-55.

[15] Khakpor A. Bibalani G.H. Mahdavi K. 2015. Optimal treatment increased the seed germination of Salvia verticillata L. J. BioSci. Biotechnol., 4(3): 255-262.

[16] Tıpırdamaz R. Gömürgen A.N. 2000. The effects of temperature and gibberellic acid on germination of Eranthis hyemalis (L.) Salisb. seeds. Turk.J. Bot., 24(1):143-145.

[17] Greipsson S. 2001. Effects of stratification and GA3 on seed germination of a sand stabilising grass Leymus arenarius used in reclamation. Seed Sci. Technol., 29: 1-10.

[18] Nasiri M. 1985. Investigation of effect divers factors on break seed dormancy White cotton. Journal of Pajohesh and Sazandegi, 28: 42-47.

[19] Sarmadnia G.H. 1996. Seed technology. Mashhad University, 288.