Seed germination of five sage species (*Salvia* sp.) of populations native to Greece

E. Kanellou, G. Vlachou, A.N. Martini, K.F. Bertsouklis and M. Papafotiou[a]

Laboratory of Floriculture and Landscape Architecture, Department of Crop Science, School of Plant Sciences, Agricultural University of Athens, Iera Odos 75, 118 55 Athens, Greece.

Abstract

In the present study, the effect of seed pretreatment and incubation temperature, on seed germination rate of five Mediterranean sage species *S. fruticosa*, *S. officinalis*, S. *pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa* was examined. Seeds of all five species were collected from native populations in August 2019 and stored at room conditions, in darkness, for 5 months. The seeds were placed for germination at constant temperatures of 10, 15, 20 and 25°C, under darkness, either without pretreatment (control) or after chemical surface scarification, by immersion in dense H_2SO_4 for 15 min. Two different substrate types were used, peat: perlite 1:1, v/v and filter paper moistened with water, in Petri dishes. In *S. fruticosa*, higher seed germination rates were observed in peat:perlite at temperatures of 10 and 15°C, without seed pretreatment (33 and 23%, respectively), while the same trend was observed in Petri dishes. In *S. pomifera* ssp. *pomifera* high seed germination rates were observed in peat:perlite at temperatures of 10, 15 and 20°C, without seed pretreatment (45, 38 and 43%, respectively). At 25°C *S. fruticosa* and *S. pomifera* ssp. *pomifera* seed germination was very low (<6%) under all treatments. In the other three species very low germination rates (<13%) were generally observed under all treatments. In conclusion, lower temperatures of 10 and 15°C had a favourable effect on seed germination of *S. fruticosa* and *S. pomifera* ssp. *pomifera*, whereas in *S. officinalis*, *S. tomentosa* and *S. ringens* further investigation is required. Generally, it was observed that both on peat:perlite substrates and Petri dishes, seed germination was improved for seeds that were not pretreated with chemical scarification.

Keywords: seed pretreatment, incubation temperature, chemical scarification, native xerophytic ornamentals

INTRODUCTION

Salvia is a populous genus that includes more than 700 species spread across the world (Ewans, 1996). There is a widespread knowledge and use of several native sage species, in different countries for medicinal purposes, perfumery, as well as culinary plants (Yucel and Yilmaz, 2009; Abdollahi et al., 2012; Khakpoor et al., 2015). However, sage species can also be used as ornamentals, given that there has been an increasing interest in xeriscaping in landscaping and gardening due to the ecological, environmental, economic and aesthetic advantages.

Native Greek sage species have striking flower colours, abundant and prolonged flowering period, varied foliage in shape and colour and are drought tolerant.

Salvia fruticosa grows along coastal areas, from sea level to 700 m, in central Greece, the Peloponnese and the islands. It flowers during early spring, with white to pink flowers.

S. officinalis grows along the mountain range of Pindos and the island of Corfu and is a well know cultivar around the world.

S. pomifera ssp. *pomifera*, endemic in Crete and south Peloponnese, grows from sea level to 500 m, and has bright pink and violet flowers.

S. tomentosa grows in Macedonia and the islands of northeast Aegean. Is has a very long flowering period, with white to violet flowers and can tolerate wet and cold climate.

[a]E-mail: mpapaf@aua.gr

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Lastly, *S. ringens* grows in mountain areas, from 500 to 1900 m altitude. It flowers during summer with big (about 3.8 cm) violet flowers and can tolerate cold.

Considering the potential value of native sage species for use as commercial ornamentals, the development of propagation protocols, including optimal seed germination conditions, is necessary.

Previous research on dormancy breaking methods and optimal germination conditions for sage species, include different incubation temperatures and photoperiods and the application of seed pretreatments such as mechanical scarification, by abrasive paper or with scalpel, removing the seed coat, moist chilling, pretreatment with KNO_3 or H_2SO_4 and gibberellic acid (GA_3). Hajebi and Soltanipoor (2006) found that seed pretreatment with mechanical scarification improved the germination of *Salvia mirzayanii* and Estaji et al. (2012) also found that seed coat removal and splitting the seed in half improved the germination of *S. ieriifolia*. For *S. verticillata* scarification with sandpaper, chemical scarification with citric acid and soaking in GA_3 treatments were found to improve germination (Khakpoor et al., 2015). Abdollahi et al. (2012) also found that seed germination percentage of sage species is increased substantially after treatment with GA_3. Thanos and Doussi (1995) found that *S. pomifera* ssp. *pomifera* and *S. fruticosa* germination was maximal (70-80%) in the temperature range of 10-20°C. Dastanpoor et al. (2013) studied the effect of seed priming on the germination percentage of *S. officinalis* seeds and the highest germination (85%) occurred when the seeds were soaked for 12 h in 30°C water, followed by soaking for 24 h in 10°C water (81%).

Due to the commercial importance and varied seed germination optimal conditions of sage species, the examination of factors affecting germination is important. There is only scarce information concerning the germination protocol of *S. pomifera* ssp. *pomifera, S. fruticosa* and *S. officinalis* and to our knowledge the seed germination of *S. tomentosa* and *S. ringens* has never been studied before. The present study aimed to examine the propagation protocol by seed of these five native to Greece sage species and thus the effect of seed pretreatment and incubation temperature on seed germination was examined in laboratory tests. The results of this study are expected to facilitate the commercial production, for ornamental use, of the five sage species.

MATERIALS AND METHODS

Experimental set up

Mature seeds were collected in August 2019 from native sage populations of *S. fruticosa* growing at mount Ymittos in Attica (central Greece), *S. officinalis* and *S. ringens* growing near Arnissa in Pella (northern Greece), *S. pomifera* ssp. *pomifera* growing near Leonidio in Arcadia (southern Greece) and *S. tomentosa* growing at the island of Thasos (northern Greece). Following, the seeds were stored at room temperature in dry conditions, in darkness, for 5 months.

Prior to placement for germination, the seeds were sorted by applying light pressure to remove empty seeds and discarding visibly deformed or extremely small seeds. The germination experiments were conducted in two different substrates:peat:perlite 1:1, v/v and filter paper moistened with distilled water in Petri dishes. The treatments included four incubation temperatures of 10, 15, 20 and 25°C and two seed pretreatments: without pretreatment (control) and chemical surface scarification by immersion in dense (98%) H_2SO_4 for 15 min, followed by a thorough washing with water before the seeds were placed for germination. Seeds were placed for germination in constant darkness in both substrates (Petri dishes were wrapped in aluminium foil).

Three replicates of 50 seeds per treatment were used on peat:perlite substrate for all five sage species. Whereas on filter paper four replicates of 25 seeds per treatment were used for *S. tomentosa, S. officinalis* and *S. ringens,* four replicates of 15 seeds per treatment were used for *S. pomifera* ssp. *pomifera* and four replicates of 12 seeds per treatment were used for *S. fruticosa*, due to limited seed availability. A seed was considered germinated when a 2-mm radicle had emerged and the germinated seeds were counted every two days for 60 days.

Statistical analysis

Analysis of variance (ANOVA) was performed utilizing Statgraphics Centurion, ver. 15.2.11 (Statpoint Technologies Inc., Warrenton, VA, USA). Treatment means were separated using Fisher's protected least significant difference (LSD) at a 0.05 probability level ($p<0.05$). The two-way ANOVA of seed germination data showed significant interactions between the two factors, i.e., seed pretreatment and incubation temperature and thus data were analysed by one-way ANOVA.

RESULTS AND DISCUSSION

The treatments significantly affected the final germination rate of all sage species, on both substrates (Tables 1 and 2). The germination rate of *S. fruticosa* seeds was generally favoured at temperatures of 10 and 15°C, when no seed pretreatment was applied. For *S. fruticosa* seeds, with no pretreatment placed in peat: perlite substrate, the highest germination percentage (33%) was observed at 10°C, whereas at 15 and 20°C it was significantly lower (23 and 12%, respectively) and the lowest germination rate (2%) occurred at 25°C (Figure 1). A similar trend was observed for *S. fruticosa* seeds with no pretreatment placed on filter paper. The highest germination rate (22%) occurred at 10°C and was lower at 15 and 20°C (20 and 17%, respectively) however, with no significant differences between treatments, while no seed germination was observed at 25°C (Figure 2). The germination rate of *S. fruticosa* seeds was generally lower for chemically scarified seeds. For scarified seeds placed in peat:perlite substrate, the highest germination percentage (15%) of *S. fruticosa* occurred at 10°C, whereas at 15 and 20°C it was lower (12 and 5%, respectively) and the lowest germination rate (1%) was observed at 25°C (Figure 1). Scarified seeds of *S. fruticosa* placed on filter paper practically did not germinate at all (Figure 2).

Table 1. One-way ANOVA on the germination rate (%) in peat: perlite substrate at a 0.05 probability level ($P<0.05$) as affected by the treatment (combined effect of seed pretreatment and temperature).

Source of variation	df	F-value				
		S. fruticosa	*S. pomifera* ssp. *pomifera*	*S. officinalis*	*S. tomentosa*	*S. ringens*
Treatment	7	16.23***	21.90***	8.91***	3.36*	5.84**

*, **, *** Significant at $p<0.05$, 0.01 and 0.001 respectively, NS: non-significant, $P \geq 0.05$.

Table 2. One-way ANOVA on the germination rate (%) in filter paper substrate at a 0.05 probability level ($P<0.05$) as affected by the treatment (combined effect of seed pretreatment and temperature).

Source of variation	df	F-value				
		S. fruticosa	*S. pomifera* ssp. *pomifera*	*S. officinalis*	*S. tomentosa*	*S. ringens*
Treatment	7	20.56***	28.85***	5.80***	3.40*	7.06***

*, **, *** Significant at $p<0.05$, 0.01 and 0.001 respectively, NS: non-significant, $P \geq 0.05$.

Similarly to *S. fruticosa,* in *S. pomifera* ssp. *pomifera* seed germination rate was generally favoured by low temperatures and no seed pretreatment. For *S. pomifera* ssp. *pomifera* the highest germination percentage in peat: perlite substrate was observed for untreated seeds at 10, 15 and 20°C (45, 38 and 43%, respectively) with no significant differences, whereas at 25°C the germination rate was significantly lower (6%) (Figure 1). For untreated seeds placed on filter paper the highest germination rate (58%) occurred at 15°C and was significantly lower at 10°C (44%) and 20°C (31%), while no germination was observed at 25°C (Figure 2). The scarification treatment produced lower germination rates on both substrates and all temperatures. At 10°C the germination rate of *S. pomifera* ssp. *pomifera* scarified seeds placed on peat:perlite was 28% and was lower at 15°C (24%) and 20°C (16%) and lowest at 25°C

(3%) (Figure 1). On filter paper, the highest germination percentage of scarified seeds of *S. pomifera* ssp. *pomifera* occurred at 15°C (23%) and was lower at 10°C (17%) and practically no seed germination was observed at 20 and 25°C (Figure 2).

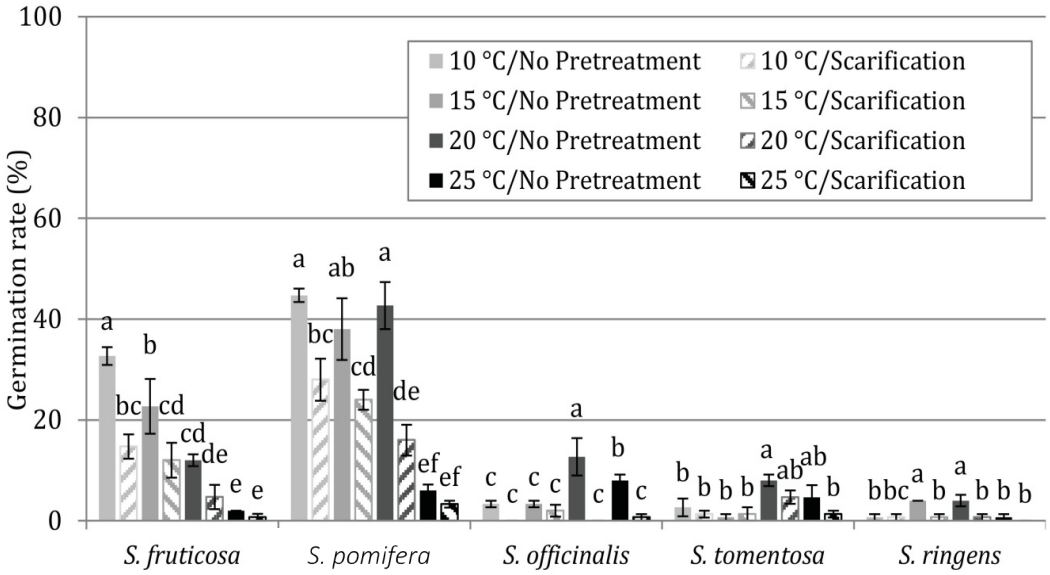

Figure 1. The effect of treatments (combined effect of seed pretreatment and temperature, one-way ANOVA) on germination rate (%) of all sage species, on the final experimental day, in peat: perlite substrate. Values are the mean of three replicates and bars represent ± standard error. Means followed by the same letter are not significantly different at $p<0.05$.

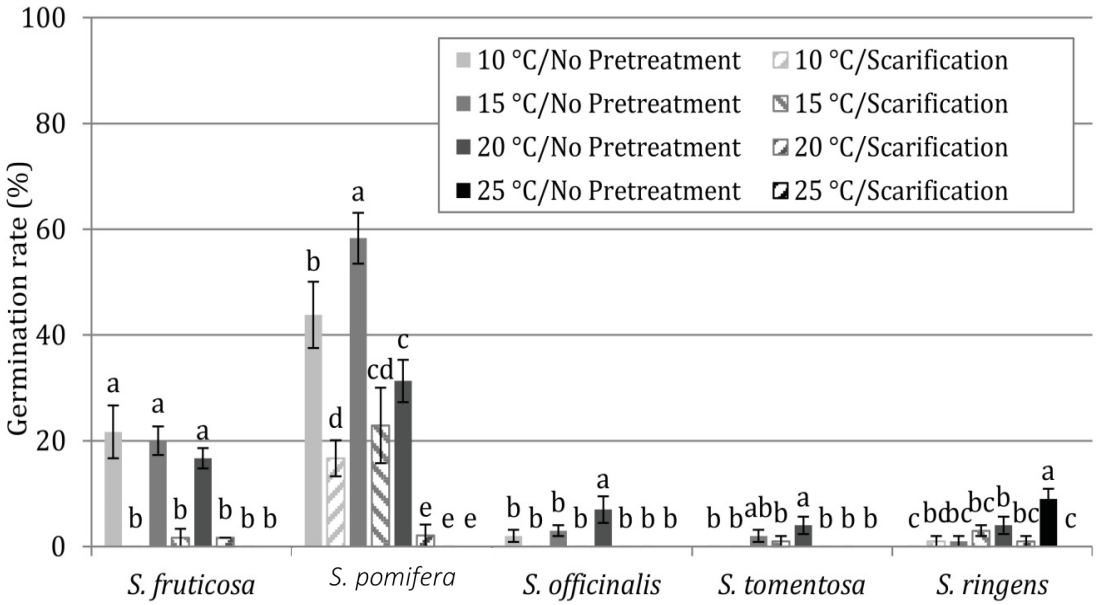

Figure 2. The effect of treatments (combined effect of seed pretreatment and temperature, one-way ANOVA) on germination rate (%) of all sage species, on the final experimental day, on filter paper substrate. Values are the mean of four replicates and bars represent ± standard error. Means followed by the same letter are not significantly different at $p<0.05$.

For *S. officinalis*, *S tomentosa* and *S ringens* very low germination rates (<13%) occurred under all treatments. For *S. officinalis* seeds, placed on peat: perlite substrate, the highest germination rate (13%) was observed at 15°C combined with no seed pretreatment and the same was observed for seeds placed on filter paper (Figures 1 and 2). Similarly *S. tomentosa* seed germination on peat: perlite substrate was highest (8%) at 15°C combined with no seed pretreatment and the same was observed for seeds placed on filter paper.

S. ringens exhibited very low germination rate under all treatments. In peat:perlite substrate only 4% of the untreated seeds germinated at 15 and 20°C (Figure 1), while on filter paper the highest germination rate (9%) occurred for untreated seeds at 25°C (Figure 2).

In our experiment, the highest germination percentages of *S. fruticosa* seeds were observed at 10°C (33%) and 15°C (23%). Thanos and Doussi (1995) reported that *S. fruticosa* seeds have an optimal temperature range for germination at 10-20°C that reached 70-80%. However, in their experiment seed germination percentage was corrected for sound seeds, as ungerminated seeds were examined under microscope and unfilled or insect infected seeds were not counted. In our experiment prior to placement for germination an effort was made to sort out empty seeds, by applying light pressure, however some unfilled or insect infected seeds may have still been placed for germination and different sorting methods may account for the difference in the final germination percentage.

Based on our results for *S. pomifera* ssp. *pomifera* the optimal temperature range is 10-20°C, which agrees with the results reported by Thanos and Doussi (1995). Similarly to *S. fruticosa* results, the highest germination rate of *S. pomifera* ssp. *pomifera* in our experiment was 38-45% and was lower than the percentages reported by Thanos and Doussi (1995), which reached 60-70%. As mentioned above the different sorting methods between the two experiments, may account for the difference in the final germination percentage.

The other three species, *S. officinalis*, *S. tomentosa* and *S. ringens* had very low (<13%) germination rates under all treatments. Dastanpoor et al. (2013) showed that seed priming by soaking seeds in sterilized water for different time intervals significantly increased seed germination of *S. officinalis* up 85%. Therefore, further investigation is required for optimal germination conditions for *S. officinalis*, as well as *S. tomentosa* and *S. ringens*.

Generally, it was observed that both in peat: perlite and Petri dishes, seed germination was improved for seeds that were not pretreated with chemical scarification. This suggests an adverse impact of the acid on seed tissue.

CONCLUSIONS

In this study, it was shown that lower temperatures of 10 and 15°C had a favourable effect on seed germination of *S. fruticosa* and *S. pomifera* ssp. *pomifera*. To improve seed germination rate of *S. officinalis*, *S. tomentosa* and *S. ringens* a broader range of methods should be tested. Chemical scarification with H_2SO_4 did not improve the germination rate and further research on other seed pretreatments is required.

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AGRICULTURAL UNIVERSITY OF ATHENS

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Salvia-Breed-GR

SEED GERMINATION OF FIVE SAGE SPECIES (*Salvia* sp.) OF NATIVE TO GREECE POPULATIONS

Kanellou E., Vlachou G., Martini A.N., Bertsouklis K., and Papafotiou M.

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INTRODUCTION

Ecological, environmental, economic and aesthetic advantages lead to more frequent use of native species in landscaping, creating the need for:

- Propagation protocols and
- New hybrids with crossings

Salvia species studied in the research program

S. fruticosa *S. officinalis* *S. pomifera* *S. ringens* *S. tomentosa*





Aim of this study

Examine the effect of seed pretreatment and incubation temperature on seed germination of the five sage species

MATERIALS AND METHODS

Seed pretreatments

- No pretreatment (control)
- Chemical surface scarification by immersion in H_2SO_4 for 15 min

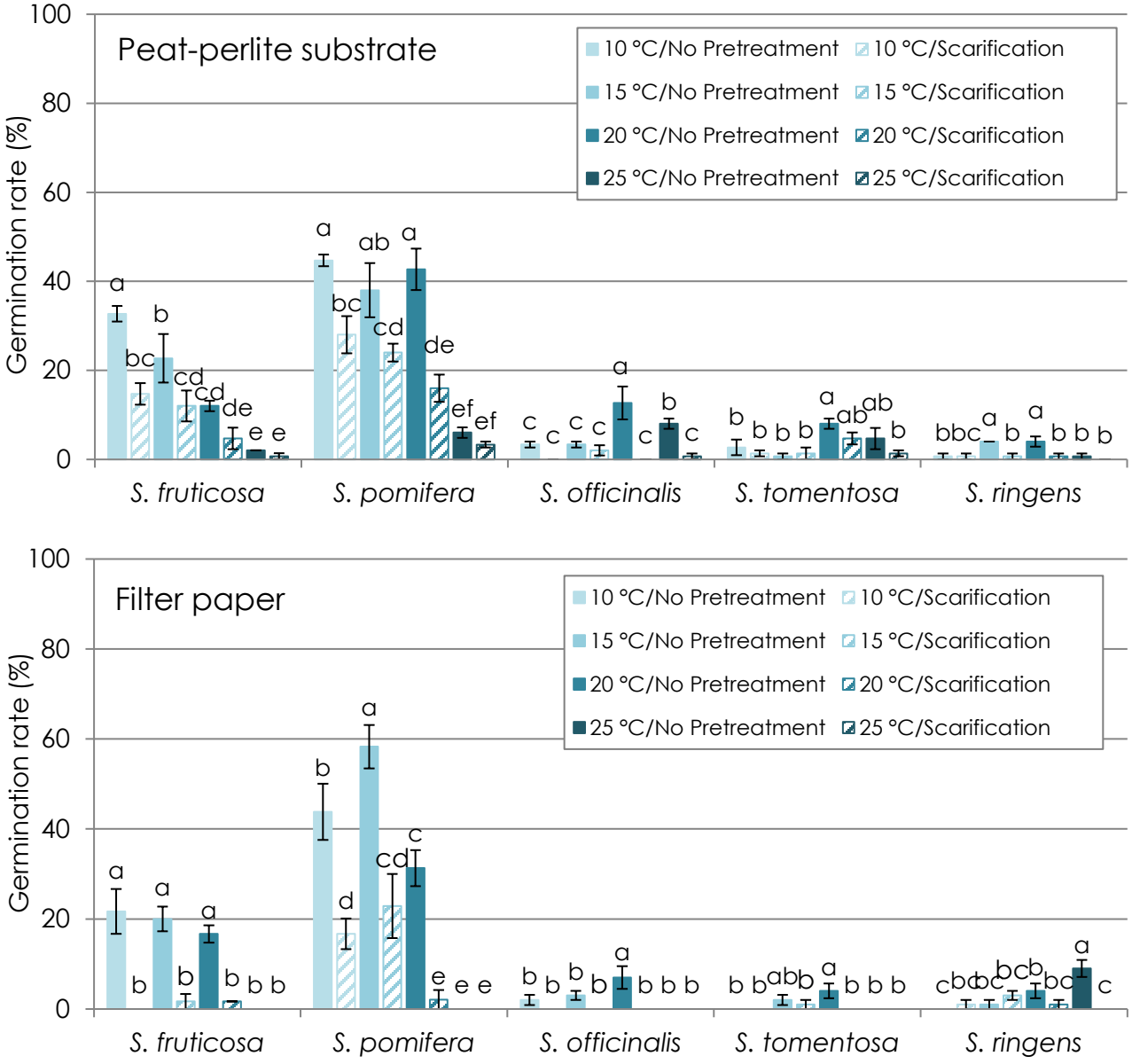
Incubation at different constant temperatures under darkness

- 10 °C
- 15 °C
- 20 °C
- 25 °C

Substrate types

- Peat-perlite substrate (1:1 v/v)
- Filter paper, moistened with water in Petri dishes

RESULTS AND CONCLUSIONS



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mpapaf@aua.gr