Comparative evaluation of seed germination of five Mediterranean sage species (*Salvia* sp.) native to Greece

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Abstract

***Salvia* sp. (*Lamiaceae*) comprises about 900 species worldwide, many of which are used as ornamental or medicinal plants. In the present study, the effect of seed pretreatment by surface scarification (mechanical or chemical) on germination of five Mediterranean sage species, *S. fruticosa*, *S. officinalis, S. pomifera* ssp. *pomifera, S. ringens*, and *S. tomentosa* was examined. Seeds were harvested from native populations in August 2018 and stored in the dark, at room temperature, for 5 months. The seeds were surface sterilized with commercial bleach solution (20% for 15 min) and placed for germination in vitro, in Petri dishes, with a solid (8 g L^{-1} agar) half-strength MS medium containing 20 g L^{-1} sucrose, at 15°C and 16-h photoperiod, either without pretreatment (control) or after scarification with sandpaper (suitable for metal surfaces) for 1 min or after dipping in dense H_2SO_4 for 15 min. In *S. fruticosa* higher seed germination percentages were observed after mechanical or chemical scarification compared to the control, whereas in *S. officinalis* dipping in H_2SO_4 was the most effective pretreatment. In the other three species, very low germination percentages (<28%) were generally observed irrespectively of pretreatment. In all species, seeds immersed in dense H_2SO_4 reached faster T_{50} than those that received mechanical scarification or no pretreatment. In conclusion, pretreatment by mechanical or chemical scarification had a favourable effect on seed germination of *S. fruticosa* and *S. officinalis*, whereas in *S. pomifera* ssp. *pomifera*, *S. tomentosa* and *S. ringens* further investigation is required.**

Keywords: seed pretreatment, scarification, in vitro seed germination, native xerophytic ornamentals

INTRODUCTION

The genus *Salvia*, is the largest of the *Lamiaceae* family and comprises about 900 species worldwide, many of which are used as ornamental or medicinal plants (Kamatou et al., 2010). The name *Salvia* comes from the Latin verb "salvare" (= save lives) (https://bit.ly/2Swt2eK). The common name "sage" is attributed to different species of the genus *Salvia*, which are widely used as ornamental or medicinal plants (Vidic et al., 2010). In Greece, there are 30 taxa (species and subspecies) of the genus *Salvia* (Dimopoulos et al., 2013), two of which are commercially known, *Salvia fruticosa* and *S. officinalis*.

The species *S. fruticosa* (Figure 1a, f) is a perennial evergreen shrub, up to 1.20 m high, which grows mainly in bushy rocky areas, often on coastal cliffs. It is endemic to the Mediterranean zone with a wider distribution from Sicily to Israel (Thanos and Doussi, 1995). In Greece, it is found in central Greece, the Peloponnese and the Aegean islands.

Salvia officinalis (Figure 1b, f), sage, is a strongly aromatic, perennial grayish shrub, up to 60 cm, which is found in garrigue, rocky pastures, scrub, rocky places (Irina, 2008). It is one of the most important species of the genus *Salvia* worldwide, as it is cultivated in many cultivars as medicinal and ornamental.

Salvia pomifera is an endemic species of the eastern Mediterranean, while the

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subspecies of *S. pomifera* ssp. *pomifera* (Figure 1c, f) is a shrub, up to 1.0 m high, that grows in dry, sunny places with brushwood vegetation and on rocky hillsides in Crete and in the Peloponnese (Thanos and Doussi, 1995).

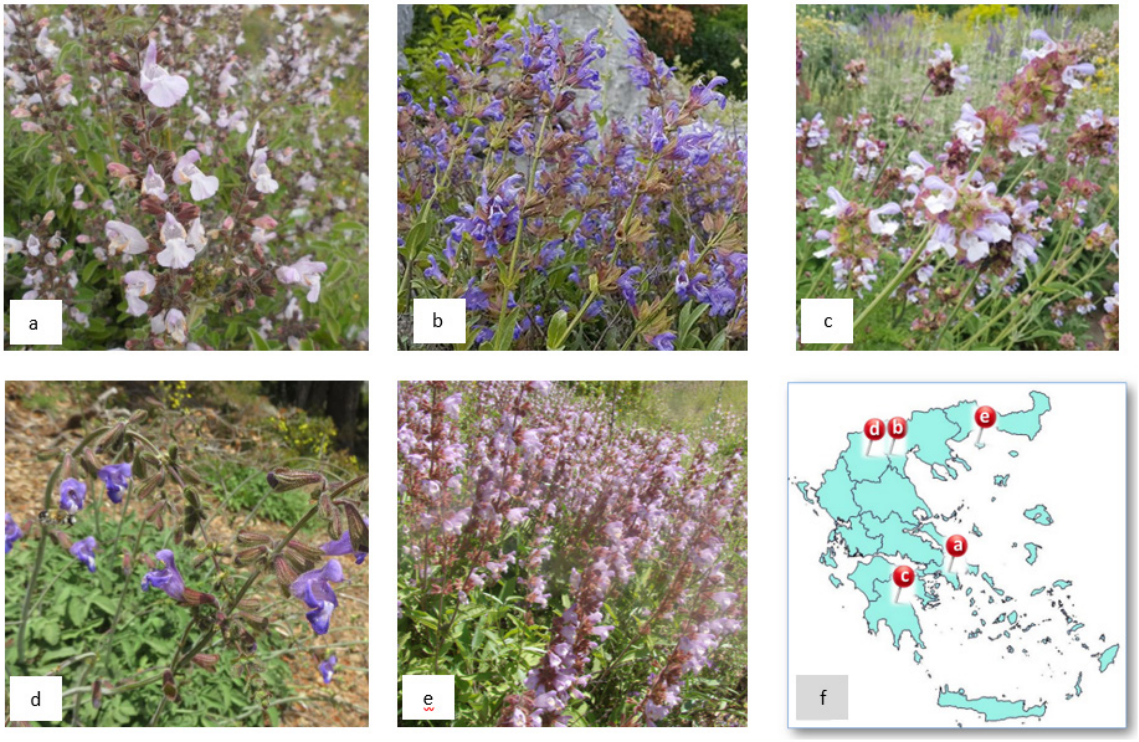

Figure 1. Plants of *S. fruticosa* (a), *S. officinalis* (b), *S. pomifera* ssp. *pomifera* (c), *S. ringens* (d) and *S. tomentosa* (e) during the flowering stage, in the wild, and seed harvesting areas for the present work (f).

Salvia ringens (Figure 1d, f) is a perennial plant with a slightly woody base and low vegetation, found in the southern and eastern parts of the Balkan Peninsula. In Greece it is spreading to the highlands of Macedonia, Epirus and central Greece. It is found mainly in areas with maquis vegetation, forest glades and streams (https://bit.ly/34zChws and https://bit.ly/2NEiWDm).

Salvia tomentosa (Figure 1e, f) is a perennial semi-woody herbaceous plant (Hedge, 1982), up to 80 cm, which grows in areas of maquis vegetation and on limestone slopes. Its geographical distribution extends from southeastern Europe to Transcaucasia (Guner et al., 2000; Euro + Med 2010 – https://bit.ly/2NiZnRT). In Greece, it spreads in northeastern Greece, in the north of central Greece, in central Greece, the northeastern, and eastern Aegean Islands (Dimopoulos et al., 2013).

Flowering of the genus *Salvia* begins in March and ends in June (depending on climatic conditions and altitude) and lasts about one month (Karamanos, 2000). The seed is smooth, oval in shape, about 3 mm in diameter and dark brown (Mabberley, 1997).

As part of the research program SALVIA-BREED-GR (https://www.salvia-breed-gr.com/el/), aiming to the improvement and promotion of Greek sage species for ornamental use, in the present study, the effect of seed pretreatment by surface scarification (mechanical or chemical) on the in vitro germination of five native Mediterranean sage species, *S. fruticosa, S. officinalis, S. pomifera* ssp. *pomifera, S. ringens*, and *S. tomentosa* was studied.

MATERIALS AND METHODS

Seeds of species *S. fruticosa, S. officinalis, S. pomifera* ssp. *pomifera*, and *S. ringens* were harvested from native populations (Figure 1) in August 2018 and stored in the dark, at room

temperature, for 5 months. The seeds were surface sterilized with commercial bleach solution (20%) (4.6% w/v NaClO) containing 1-2 drops of Tween 20 (polyxyethylenesorbitan monolaurate, MERCK) for 15 min, rinsed four times (3 min each) with sterile distilled water and put to germinate in 9-cm Petri dishes with 20 mL of solid (8 g L^{-1} agar), half-strength MS medium (Murashige and Skoog, 1962) at 15°C and 16 h cool white fluorescent light (37.5 µmol m^{-2} s^{-1})/8-h dark photoperiod,either without pretreatment (control) or after scarification with sandpaper (suitable for metal surfaces) for 1 min or after dipping in dense H_2SO_4 for 15 min. Germination was defined as the appearance of the radicle at least 2 mm long (Figure 2) according to the rules of the International Seed Testing Association (1999). T_{50} is defined as time for 50% germination of seeds.

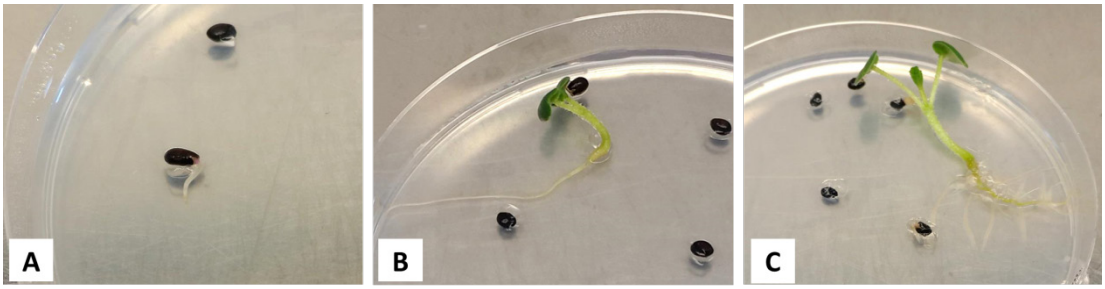

Figure 2. Seed germination stages of *Salvia* sp. in vitro, in Petri dishes, with solid half-strength MS medium: germination (A), root and hypocotyl elongation (B), shoot elongation and root branching (C).

The completely randomized design was used in all experiments and the significance of the results was tested by one-way analysis of variance (ANOVA). The means of the treatments were compared by the Student's *t* test at p=0.05 (JMP 11.0 software, SAS Institute Inc., Cary, NC, 2013, USA).

RESULTS AND DISCUSSION

In *S. fruticosa,* higher seed germination percentages were observed after scarification with sandpaper (suitable for metal surfaces) (79%) or after dipping in dense H_2SO_4 (84%) compared to the control (56%) (Figure 3).

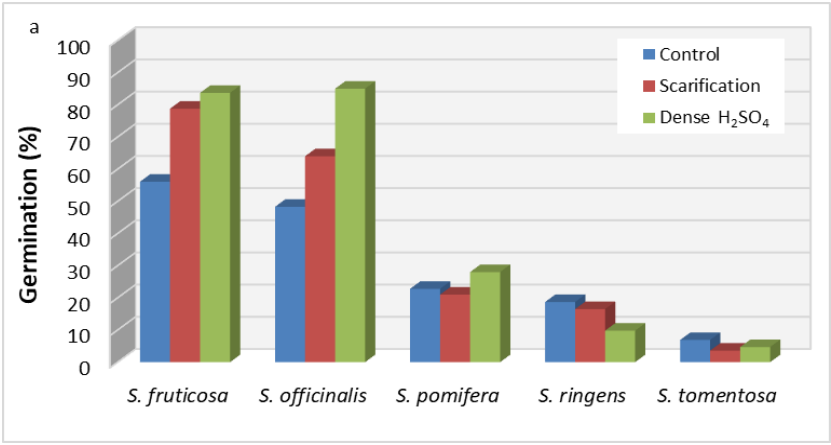

Figure 3. In vitro germination of seeds of the species *S. fruticosa, S. officinalis, S. pomifera* ssp. *pomifera, S. ringens*, and *S. tomentosa,* 5 months after harvesting, at 15°C, either without pretreatment (control) or after scarification with sandpaper (suitable for metal surfaces) for 1 min or after dipping in dense H_2SO_4 for 15 min.

In *S. officinalis,* dipping in dense H_2SO_4 was the most effective pretreatment (85%), followed by scarification with sandpaper (64%) and control (48%) (Figure 3).

In the other three species, very low germination percentages (<28%) were generally observed irrespectively of pretreatment, 21-28% in *S. pomifera* ssp. *pomifera,* 4-7% in *S. tomentosa* and 9-19% in *S. ringens* (Figure 3).

Regarding germination rapidity, in all species, seeds dipped in concentrated H_2SO_4 for scarification reached T_{50} earlier (20-42 days) than those scarified with sandpaper (26-62 days) or those that were not scarified (control) (48-94 days) (Figure 4).

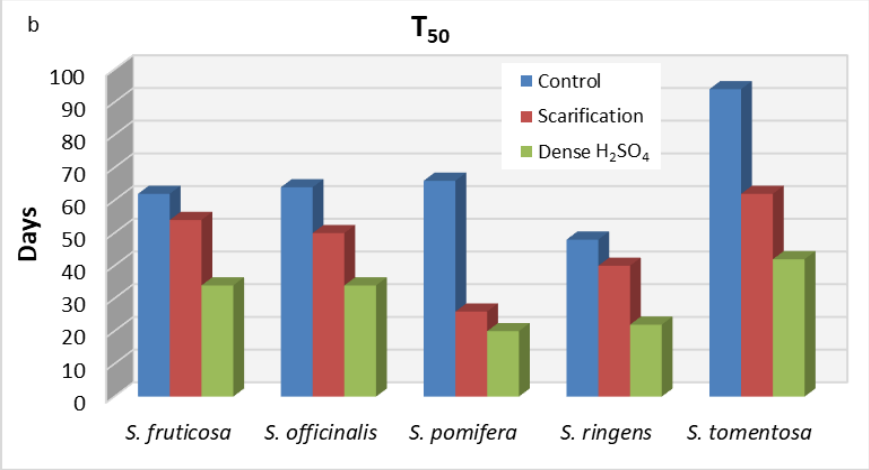

Figure 4. Rapidity of seed germination (T_{50}) in vitro of the species *S. fruticosa, S. officinalis, S. pomifera* ssp. *pomifera, S. ringens*, and *S. tomentosa,* 5 months after harvesting, at 15°C, either without pretreatment (control) or after scarification with sandpaper (suitable for metal surfaces) for 1 min or dipping in dense H_2SO_4 for 15 min. T_{50} is defined as time for 50% germination of seeds.

The above mentioned results demonstrate the emergence of a natural dormancy due to the hard and impermeable seed coat of the species *S. fruticosa* and *S. officinalis*, similarly to results found by Aghilian et al. (2014) for *S. officinalis*. In addition, this behaviour is consistent with the behaviour of other species of the genus *Salvia*, such as *S. leriifolia* Bent. (Nuruozak), *S. verticillata* (Khakpor et al., 2011), *S. stenophylla* (Musarurwa et al., 2010), *S. dorrii* (Aghilian et al., 2014), *S. spinosa*, *S. chloreleuca*, *S. multicaulis*, *S. hydrangea* and *S. sharifii*, *S. sclarea* (Ellis et al., 1985), *S. glutinosa* (Thompson, 1969), *S. mellifera* (Keeley, 1986; Emery, 1988; Thanos and Rundel, 1995), *S. columbariae* (Capon and Brecht, 1970; Hashemi and Estilai, 1994), *S. cyanescens* Boiss. & Bal. (Yücel and Yilmaz, 2009). As regards the species *S. pomifera* ssp. *pomifera*, *S. tomentosa*, and *S. ringens*, further investigation is required to determine the cause of low germination rate.

CONCLUSIONS

Pretreatment by mechanical (with sandpaper) or chemical (dense H_2SO_4) scarification had a promoting effect on seed germination of *S. fruticosa* and *S. officinalis*. Concerning germination of the species *S. pomifera* ssp. *pomifera*, *S. tomentosa*, and *S. ringens*, further investigation is required.

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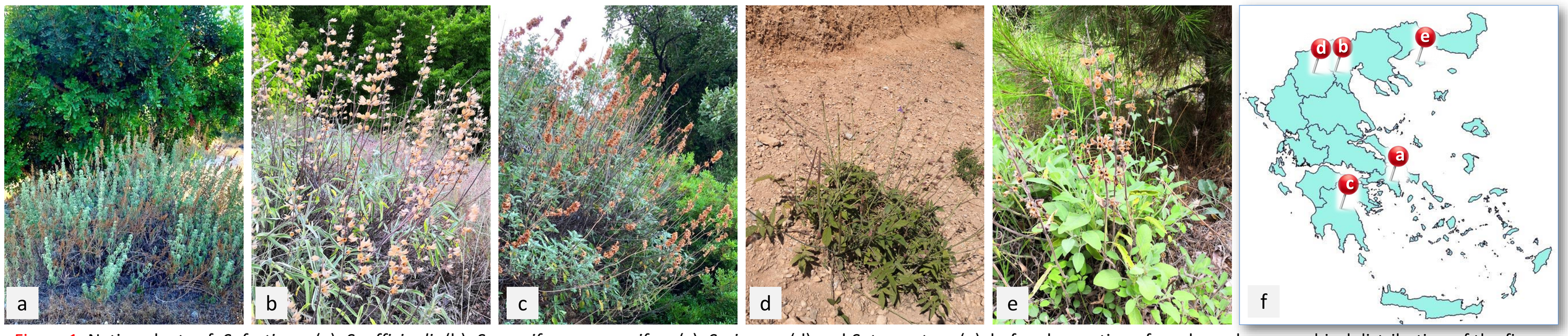

Figure 1. Native plants of *S. fruticosa* (a), *S. officinalis* (b), *S. pomifera* ssp *pomifera* (c), *S. ringens* (d) and *S. tomentosa* (e), before harvesting of seeds, and geographical distribution of the five species in Greece (f).

Introduction

The genus Salvia, is the largest of the Lamiaceae family and comprises about 900 species worldwide, many of which are used as ornamental or medicinal plants (Kamatou *et al.*, 2010). The name Salvia comes from the Latin verb ¨salvare¨ (= save lives) (https://bit.ly/2Swt2eK). The common name «sage» is attributed to different species of the genus *Salvia*, which are widely used as ornamental or medicinal plants (Vidic and Maksimović, 2010). In Greece, there are 30 taxa (species and subspecies) of the genus Salvia (Dimopoulos et al., 2013), two of which are commercially known, *Salvia fruticosa* and *S. officinalis*.

The seed is smooth, oval in shape, about 3 mm in diameter and dark brown (Mabberley, 1997) (Figure 2).

As part of the research program SALVIA-BREED-GR (https://www.salvia-breed-gr.com/el/), aiming to the improvement and promotion of Greek sage species for ornamental, in the present study, the effect of seed pretreatment by surface scarification (mechanical or chemical) on the *in vitro* germination of five native Mediterranean sage species, *S. fruticosa, S. officinalis, S. pomifera* ssp *pomifera, S. ringens* and *S.* tomentosa was examined. (Figure 1).

Figure 2. Typical seeds of the genus *Salvia.*

Materials and Method

Seeds of species *S. fruticosa, S. officinalis, S. pomifera* ssp *pomifera* and *S. ringens* were harvested from native populations in August 2018 and stored in the dark, at room temperature, for 5 months, with the exception of those of *S. tomentosa* harvested from plants grown in Eastern Attica. The seeds were surface sterilized with commercial bleach solution (20%) (4.6% w/v NaClO) for 15 min and placed for germination *in vitro*, in Petri dishes, with a solid (8 g L^{-1} agar) half-strength MS medium containing 20 g L^{-1} sucrose, at 15 ° C and 16 h photoperiod at 37.5 μmol m^{-2} s^{-1}, provided by cool-white fluorescent lamps, either without pretreatment (control) or after scarification with sandpaper (suitable for metal surfaces) for 1 min or after dipping in dense H_2SO_4 for 15 min. Germination was defined as the appearance of a radicle at least 2 mm long according to the rules of the International Seed Testing Association (1999). T_{50} = is defined as time for 50% germination of seeds.

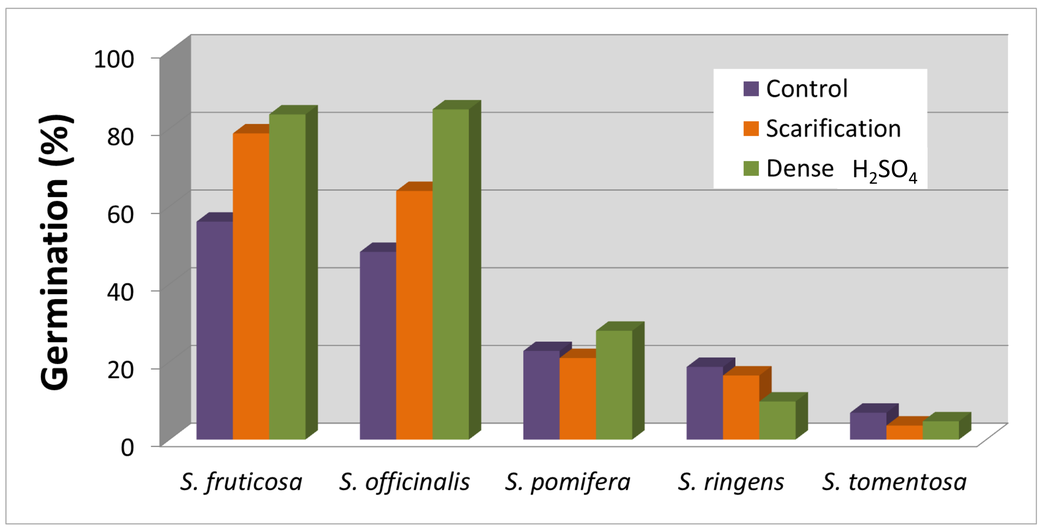

Figure 3. *In vitro* germination of seeds of the species *S. fruticosa, S. officinalis, S. pomifera* ssp *pomifera, S. ringens* and *S. tomentosa,* 5 months after harvesting, at 15 °C, either without pretreatment (control) or after scarification with sandpaper (suitable for metal surfaces) for 1 min or after dipping in dense H_2SO_4 for 15 min.

Results

In *S. fruticosa,* higher seed germination percentages were observed after scarification with sandpaper (suitable for metal surfaces) (79%) or after dipping in dense H_2SO_4 (84%) compared to the control (56%) (Figure 3, Figure 5).

In *S. officinalis,* dipping in dense H_2SO_4 was the most effective pretreatment (85%), followed by scarification with sandpaper (64%) and control (48%) (Figure 3, Figure 5).

In the other three species, very low germination percentages (<28%) were generally observed irrespectively of pretreatment, 21-28% in *S. pomifera* ssp. *pomifera,* 4-7% in *S. tomentosa* and 9-19 % in *S. ringens* (Figure 3, Figure 5).

Regarding germination rapidity, in all species, the seeds dipped in concentrated H_2SO_4 reached T_{50} earlier (20-42 days) than those that received scarification with sandpaper (26-62 days) or no pretreatment (control) (48-94 days) (Figure 4).

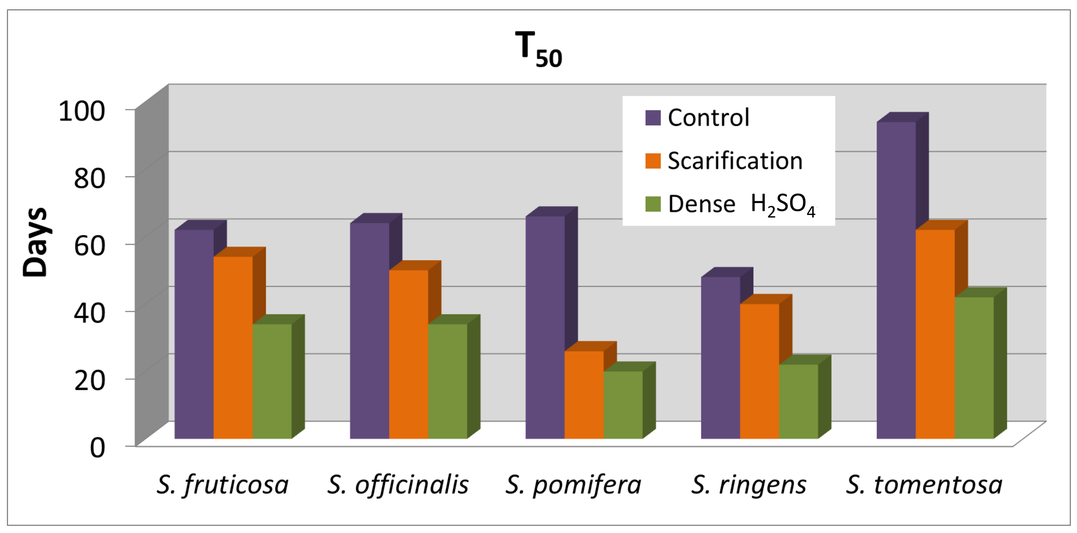

Figure 4. Rapidity of seed germination (T_{50}) *in vitro* of the species *S. fruticosa, S. officinalis, S. pomifera ssp pomifera, S. ringens* and *S. tomentosa,* 5 months after harvesting, at 15 °C, either without pretreatment (control) or after scarification with sandpaper (suitable for metal surfaces) for 1 min or after dipping in dense H_2SO_4 for 15 min.
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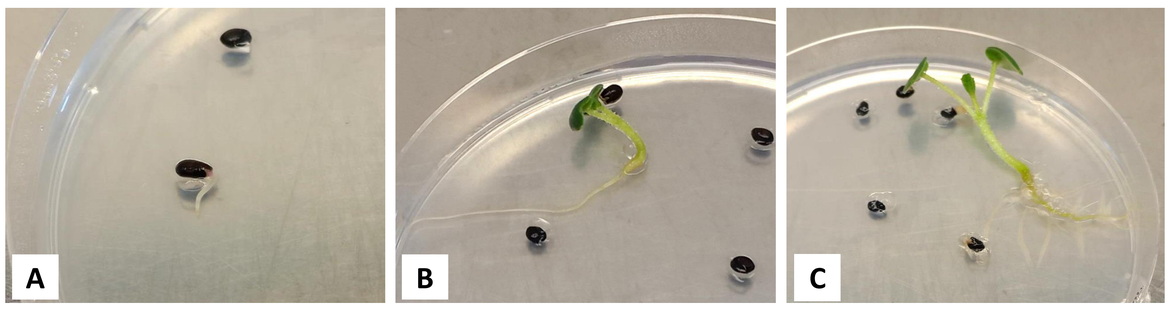

Figure 5. Seed germination stages of *Salvia* sp. *in vitro,* in Petri dishes, with solid MS) half-strength MS medium: germination initiation (A), shoot elongation (B), shoot elongation and root elongation (C).

Conclusions

- ✓ Pretreatment by mechanical (with sandpaper) or chemical scarification (dense H_2SO_4) had a favorable effect on seed germination of *S. fruticosa* and *S. officinalis*.
- ✓ For the species *S. pomifera* ssp. *pomifera*, *S. tomentosa* and *S. ringens* further investigation is required.

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