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

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

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, we examined the effect of application method and concentration of indole-3-butyric acid (IBA) on the rooting of stem cuttings of five wild species of sage from Greece: *Salvia fruticosa*, *S. officinalis*, *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa*. The aim was to find an effective rooting method, along with obtaining mother plants and plant-clones with desirable characteristics for future crosses to improve wild species for potential horticultural use. Shoot-tip cuttings, 12-15 cm long, collected from native plants in April to May 2019, were treated either with dusting powder Rhizopon (0.5% w/w IBA) or their bases were dipped for 1 min in solutions of IBA (50% ethanol) at 0 (control) or 500 or 1000 or 2000 or 3000 (mg L^{-1}). The cuttings were then placed for rooting on peat-perlite (1:1 v/v) in a mist for 2 weeks, and then remained on the greenhouse bench in a semi-shaded location for another 4 weeks. In all species, with the exception of *S. officinalis*, higher rooting (over 80%) of cuttings were observed after treatment with dusting powder or immersion in a 2000 or 3000 mg L^{-1} IBA solution compared to immersion in lower concentrations of IBA or the control. In *S. officinalis*, lower rooting percentages were generally observed compared to other species, probably because cuttings were in bloom and not sufficiently lignified. The best treatments for this species were immersion in a solution of 2000 or 3000 mg L^{-1} IBA, providing about 50% rooting.

Keywords: indole-3-butyric acid (IBA), shoot-tip cuttings, rooting, native xerophytic ornamentals

INTRODUCTION

Mediterranean *Salvia* species are ideal for xeriscape landscaping, due to their reduced water and cultivation requirements, as well as their high ornamental value (rich inflorescence and colour diversity of flowers). They are important plants for bees too. *Salvia fruticosa*, called 'faskomelo' in Greek, grows up to 1.20 m high and has white felted stems. Its leaves often have 1-2 pairs of small lobes below the main one, and the flowers have high colour variability (lilac, pink or sometimes white) in March-June. The leaves are used for flavoring and for an herbal tea (Blamey and Grey-Wilson, 1993). It is endemic to the Mediterranean zone with a wider distribution from Sicily to Israel (Thanos and Doussi, 1995). It shows high adaptability to drought.

Salvia officinalis is a strongly aromatic, rather grayish shrub up to 60 cm tall, with branches spreading to erect, becoming woody below, leaves oblong to elliptical that are rough greenish above but white felted beneath, with a margin that is finely toothed. Its flowers are violet, blue, pink or white in May-July. The calyx is often flushed with purple. The species prefers garrigue, stony pastures, scrub, and rocky places in Spain, S. France and the Balkans. It is also widely grown and naturalized elsewhere (Tutin et al., 1972; Blamey and Grey-Wilson, 1993). It is cultivated worldwide with many cultivars for pharmaceutical and ornamental uses.

Salvia pomifera is an endemic species of the eastern Mediterranean. It is found in

[a]E-mail: martini_agr@yahoo.com

southern Greece and the Aegean islands and has oval leaves and violet-blue flowers and with a much enlarged calyx, often reddish-purple in colour (Tutin et al., 1972; Blamey and Grey-Wilson, 1993). The subspecies *S. pomifera* ssp. *pomifera* grows in dry, sunny places with phrygian vegetation and on rocky hillsides in Crete and in the Peloponnese (Thanos and Doussi, 1995). It has pink and violet flowers of intense colour.

Salvia ringens grows up to 0.60 m high, is woody at its base, has leaves that are pinnatisect or pinnate with 3-6 pairs of small lateral segments. The flowers are appressed-hairy and violet-blue or blue. The species is distributed in the southern and eastern parts of the Balkan Peninsula (Tutin et al., 1972). It has low vegetation habit and develops tall, branching flowering stems with large violet flowers during the spring and summer. The plant is resistant to low temperatures.

Salvia tomentosa Miller (synonym *S. grandiflora*) is similar to *S. officinalis*, but has leaves with a rounded or heart-shaped base (Blamey and Grey-Wilson, 1993). Its geographical distribution extends from southern Europe (mostly Balkan Peninsula and Crimea) and part of western Asia (Anatolia and Near East), in areas of maquis vegetation and on limestone slopes (Guner et al., 2000). It shows intense variability in flower colour (from white to violet) and plant size, has a particularly long flowering period, is drought tolerant, and adaptable to wet conditions and resistance to low temperatures.

The asexual propagation by stem cuttings is a simple and easily applied method of plant propagation, which is preferred in case of medicinal and aromatic herbs rather than propagation by seeds, because of their low seed germination capacity (lower than 50%) and the lack of seed selection programs (Nicola et al., 2005). There are several reports on rooting cuttings of *S. officinalis* (Nicola et al., 2003, 2005; Kaçar et al., 2009; Capecka, 2012; Paradiković et al., 2013; Gudeva et al., 2017) and *S. frusicosa* (Sağlam et al., 2014), while rooting of *S. pomifera* ssp. *pomifera*, *S. ringens*, and *S. tomentosa* cuttings has not been studied yet. Experimentation for each specific plant is necessary in order to determine the appropriate rooting hormone treatment, since it is well established that exogenous application of auxin accelerates the rates of rooting, increases final rooting percentage and improves the number of produced roots in leafy cuttings (De Klerk et al., 1999).

As part of a research program (SALVIA-BREED-GR) on the improvement and promotion of Greek sage species for ornamental use, the effect of application method and concentration of indole-3-butyric acid (IBA) on the rooting of stem cuttings of five wild species of sage found in Greece was investigated. We aimed to find an effective rooting method to enable the production of mother plants and plant-clones with desirable characteristics for future crosses.

MATERIALS AND METHODS

Five sage species found in Greece were studied: *Salvia fruticosa*, *S. officinalis*, *S. pomifera* ssp. *pomifera*, *S. ringens*, and *S. tomentosa.*

Shoot-tip cuttings (Figure 1), 12-15 cm long, were collected in early April 2019 for the species of central and southern Greece (*Salvia fruticosa* and *S. pomifera* ssp. *pomifera*) and in early May for the species of Northern Greece (*S. officinalis*, *S. ringens*, and *S. tomentosa*)

Cuttings were treated either with dusting powder (Rhizopon (containing 0.5% w/w IBA)) or their bases were dipped for 1 min in solutions of IBA (in 50% ethanol) with concentrations of 0 (control) or 500 or 1000 or 2000 or 3000 mg L^{-1}. Then the cuttings were placed for rooting on peat-perlite 1:1 (v/v) in a mist for 2 weeks. Thereafter, they remained on the greenhouse bench in a semi-shaded location for another 4 weeks (Figure 2).

A completely randomized design and four repetitions of ten cutting per treatment were used; the significance of the results was tested by one- or two-way analysis of variance (ANOVA) and treatment means were compared by Student's *t* test at $p \leq 0.05$.

Figure 1. Typical shoot-tip cuttings collected in spring of the species *Salvia fruticosa* (a), *S. officinalis* (b), *S. pomifera* ssp. *pomifera* (c), *S. ringens* (d), and *S. tomentosa* (e).

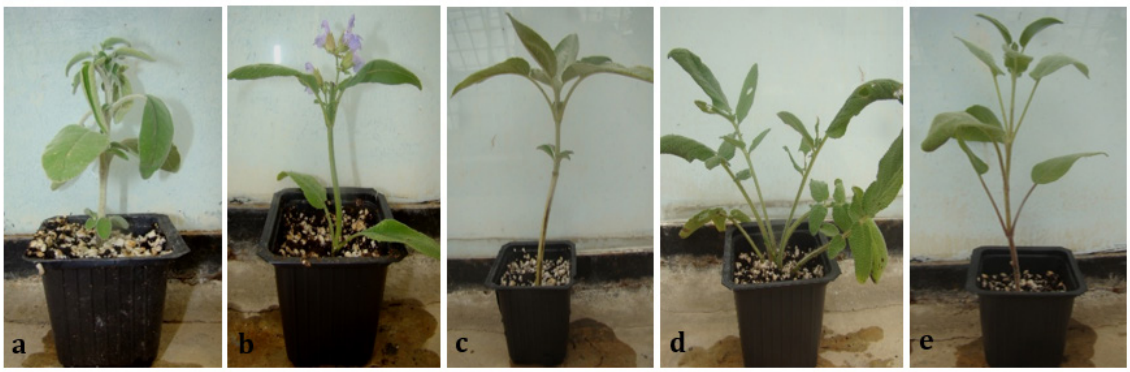

Figure 2. Rooted cuttings of the species *Salvia fruticosa* (a), *S. officinalis* (b), *S. pomifera* ssp. *pomifera* (c), *S. ringens* (d), and *S. tomentosa* (e).

RESULTS AND DISCUSSION

In species *S. fruticosa*, *S. pomifera* ssp. *pomifera* and *S. ringens*, higher rooting percentages of cuttings were observed after treatment with dusting powder Rhizopon or immersion in a 2000 or 3000 mg L^{-1} IBA solution compared to immersion in a solution of 500 or 1000 mg L^{-1} IBA or the control (Figure 3a, c and d). In contrast, the treatment with Rhizopon was the most effective for *S. tomentosa*, one (Figure 3e).

In *S. officinalis*, lower rooting percentages were generally observed compared to other species. Immersion in a solution of 2000 or 3000 mg L^{-1} IBA induced the highest response (Figure 3b), unlike previous reports where higher than 70% rooting percentages were achieved either using Rhizopon (Paradiković et al., 2013) or after immersion in 1000 mg L^{-1} IBA for 5 s (Kaçar et al., 2009). The poor rooting of *S. officinalis* cuttings in the present work could be attributed to the fact that they were not sufficiently lignified and at the same time they were in bloom (Figures 1b and 3b).

In previous studies on *S. officinalis* and *S. fruticosa*, hormones were shown to enhance out-of-season rooting of cuttings (Nicola et al., 2003) and had a positive effect on root system development, both in terms of root number and longest root length (Nicola et al., 2005), plant height, number of leaves, fresh weight and dry weight of plants (Paradiković et al., 2013; Sağlam et al., 2014). According to Sağlam et al. (2014), high hormone dose applications cause a notable increase in root weight and root number, while low hormone applications do not affect root length. On the other hand, Gudeva et al. (2017) showed that the vegetative propagation of medicinal and aromatic species might be stimulated by auxins utilization even in very low concentrations.

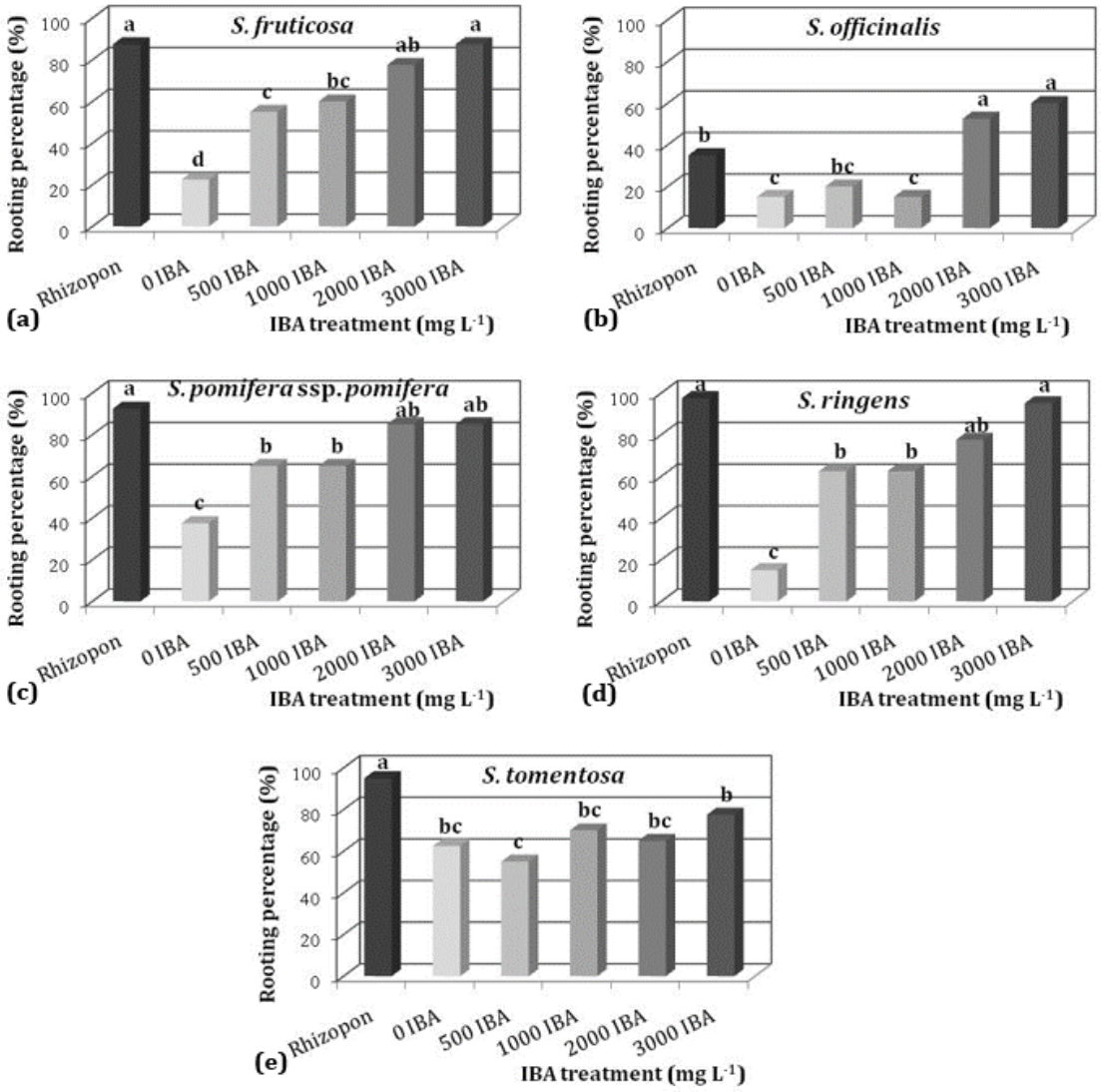

Figure 3. Effect of application method and concentrations of IBA on rooting cuttings of the species *Salvia fruticosa* (a), *S. officinalis* (b), *S. pomifera* ssp. *pomifera* (c), *S. ringens* (d), and *S. tomentosa* (e).

CONCLUSIONS

The most effective methods for rooting cuttings of the studied *Salvia* species were treatment with dusting powder Rhizopon (containing 0.5% w/w IBA) and immersion of their bases in a 2000 or 3000 mg L^{-1} IBA solution for 1 min. The methods developed will enable the easier production of mother plants and plant-clones with desirable characteristics for future crosses.

ACKNOWLEDGEMENTS

This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH - CREATE - INNOVATE (Project code:T1EDK-04923, Project: SALVIA-BREED-GR).

Literature cited

Blamey, M., and Grey-Wilson, C. (1993). Mediterranean Wild Flowers (London: Harper Collins Publishers), p.401–402.

Capecka, E. (2012). The effect of propagation term and method on the growth and fresh herb productivity of sage and balm cultivated in pots. Folia Hortic. *24* (*1*), 67–71 https://doi.org/10.2478/v10245-012-0008-y.

De Klerk, G., Van Der Krieken, W., and De Jong, J.C. (1999). Review. The formation of adventitious roots: new concepts, new possibilities. In Vitro Cell. Dev. Biol. *35* (*3*), 189–199 https://doi.org/10.1007/s11627-999-0076-z.

Gudeva, L., Trajkova, F., Mihajlov, L., and Troicki, J. (2017). Influence of different auxins on rooting of rosemary, sage and elderberry. Annu. Res. Rev. Biol. *12* (*5*), 1–8 https://doi.org/10.9734/ARRB/2017/33162.

Guner, A., Ozhatay, N., Ekim, T., and Baser, K.H.C. (2000). Flora of Turkey and the East Aegean Islands. (Supplement II), Vol. 11 (Edinburg: Edinburg University Press).

Kaçar, O., Azkan, N., and Çöplü, N. (2009). Effects of different rooting media and indole butyric acid on rooting of stem cuttings in sage (*Salvia officinalis* L. and *Salvia triloba* L.). J. Food Agric. Environ. *7*, 349–352.

Nicola, S., Fontana, E., and Hoeberechts, J. (2003). Effects of rooting products on medicinal and aromatic plant cuttings. Acta Hortic. *614*, 273–278 https://doi.org/10.17660/ActaHortic.2003.614.40.

Nicola, S., Fontana, E., Hoeberechts, J., and Saglietti, D. (2005). Rooting products and cutting timing on sage (*Salvia officinalis* L.) propagation. Acta Hortic. *676*, 135–141 https://doi.org/10.17660/ActaHortic.2005.676.16.

Paradiković, N., Zeljković, S., Tkalec, M., Vinković, T., Dervić, I., and Marić, M. (2013). Influence of rooting powder on propagation of sage (*Salvia officinalis* L.) and rosemary (*Rosmarinus officinalis* L.) with green cuttings. Poljoprivreda (Osijek) *19*, 10–15.

Sağlam, A.N., Yaver, S., Başer, I., and Cinkiliç, L. (2014). The effects of different hormones and their doses on rooting of stem cuttings in Anatolian sage (*Salvia fruticosa* Mill.). APCBEE Procedia *8*, 348–353 https://doi.org/10.1016/j.apcbee.2014.03.052.

Thanos, C.A., and Doussi, M.A. (1995). Ecophysiology of seed germination in endemic labiates of Crete. Isr. J. Plant Sci. *43* (*3*), 227–237 https://doi.org/10.1080/07929978.1995.10676607.

Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., and Webb, D.A. (1972). Flora Europaea. Vol. 3 *Diapenstaceae* to *Myoporaceae* (UK: Cambridge University Press), p.188–190.

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

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

Agricultural University of Athens, Department of Crop Science, Laboratory of Floriculture and Landscape Architecture, Iera Odos 75, 118 55 Athens, Greece, E-mail: martini_agr@yahoo.com

Introduction

Mediterranean Salvia species are ideal for xeriscape landscaping, due to their reduced water and cultivation requirements, as well as their high ornamental value (rich inflorescence and color diversity of flowers) (Figure 1).

As part of the research program SALVIA-BREED-GR*, which concerns the improvement and promotion of Greek sage species for ornamental use, in the present study, the effect of application method and concentration of indole-3-butyric acid (IBA) on rooting stem cuttings of five Greek wild species of sage, *Salvia fruticosa*, *S. officinalis*, *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa* were examined, aiming to find an effective rooting method, along with obtaining mother plants and plant-clones with desirable characteristics for future crosses.

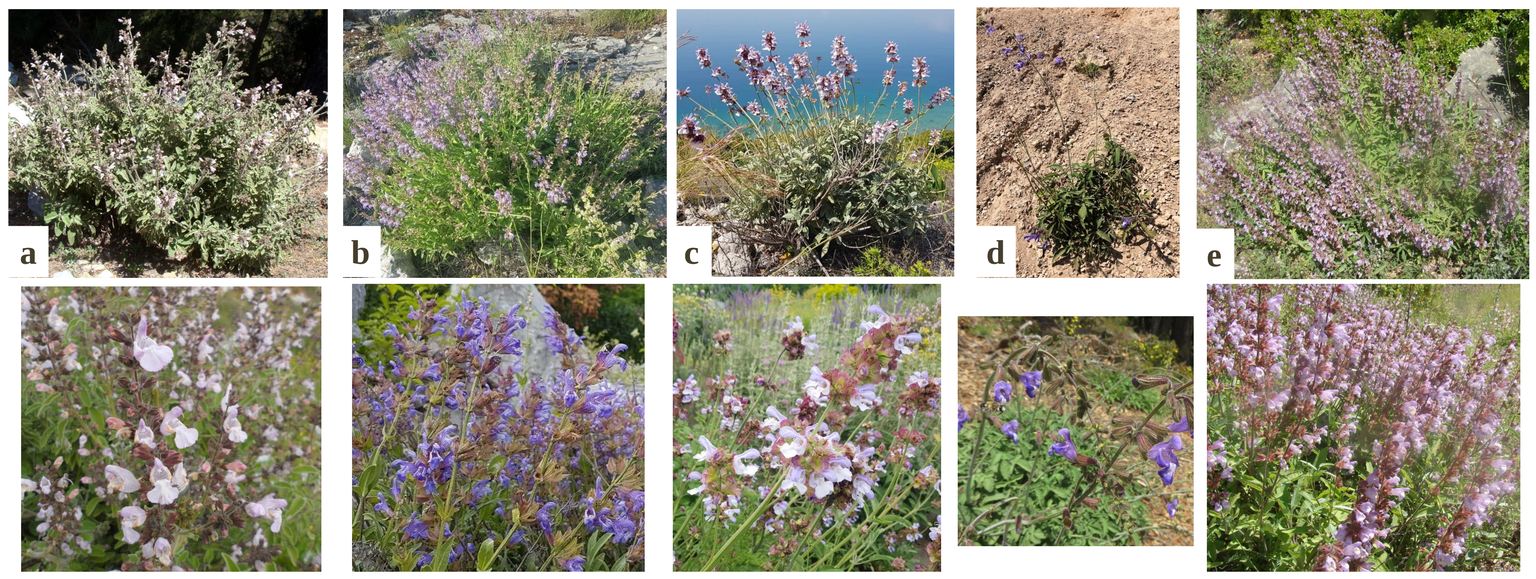

Figure 1. Native plants and characteristic inflorescence of the species *Salvia fruticosa* (a), *S. officinalis* (b), *S. pomifera* ssp. *pomifera* (c), *S. ringens* (d) and *S. tomentosa* (e).

Figure 2. Typical shoot-tip cuttings collected in spring of the species *Salvia fruticosa* (a), *S. officinalis* (b), *S. pomifera* ssp. *pomifera* (c), *S. ringens* (d) and *S. tomentosa* (e).

Results and Discussion

In species *S. fruticosa*, *S. pomifera* ssp. *pomifera* και *S. ringens*, higher rooting percentages of cuttings were observed after treatment with dusting powder Rhizopon or immersion in a 2000 or 3000 mg/L IBA solution compared to immersion in a solution of 500 or 1000 mg/L IBA or control (Figure 3a, c and d), while in *S. tomentosa*, the treatment with Rhizopon was the best treatment (Figure 3e).

In *S. officinalis*, lower rooting percentages were generally observed compared to other species, while immersion in a solution of 2000 or 3000 mg/L IBA were the best treatments for this species (Figure 3b), although high rooting percentages have been reported for this species, higher than 70%, either using Rhizopon (Paradiković *et al.*, 2013) or after immersion in 1000 mg/L IBA for 5 sec (Kaçar *et al.*, 2009). The poor rooting of *S. officinalis* cuttings in the present work could be attributed to the fact that they were not sufficiently lignified and at the same time they were all in blooming (Figures 2b and 4b).

Conclusions

The most effective methods for rooting cuttings of the studied Salvia species were treatment with dusting powder Rhizopon (0.5% w/w IBA) and immersion of their bases in a 2000 or 3000 mg/L IBA solution for 1 min.

Materials and Methods

Shoot-tip cuttings (Figure 2), 12-15 cm long, collected from native plants, in early April 2019 for the species of Central and Southern Greece, *Salvia fruticosa* and *S. pomifera* ssp. *pomifera*, and in early May for the species of Northern Greece, *S. officinalis*, *S. ringens* and *S. tomentosa*.

Cuttings were treated either with dusting powder Rhizopon (0.5% w/w IBA) or their bases were dipped for 1 min in solution IBA (50% ethanol) with concentration 0 (control) or 500 or 1000 or 2000 or 3000 mg/L and were placed for rooting on peat-perlite 1:1 (v/v) in a mist for 2 weeks. Then, they remained on the greenhouse bench in a semi-shaded location for another 4 weeks (Figure 4).

The completely randomized design and four repetitions of ten cutting per treatment were used, the significance of the results was tested by one- or two-way analysis of variance (ANOVA) and treatment means were compared by Student's *t* test at $P \leq 0.05$.

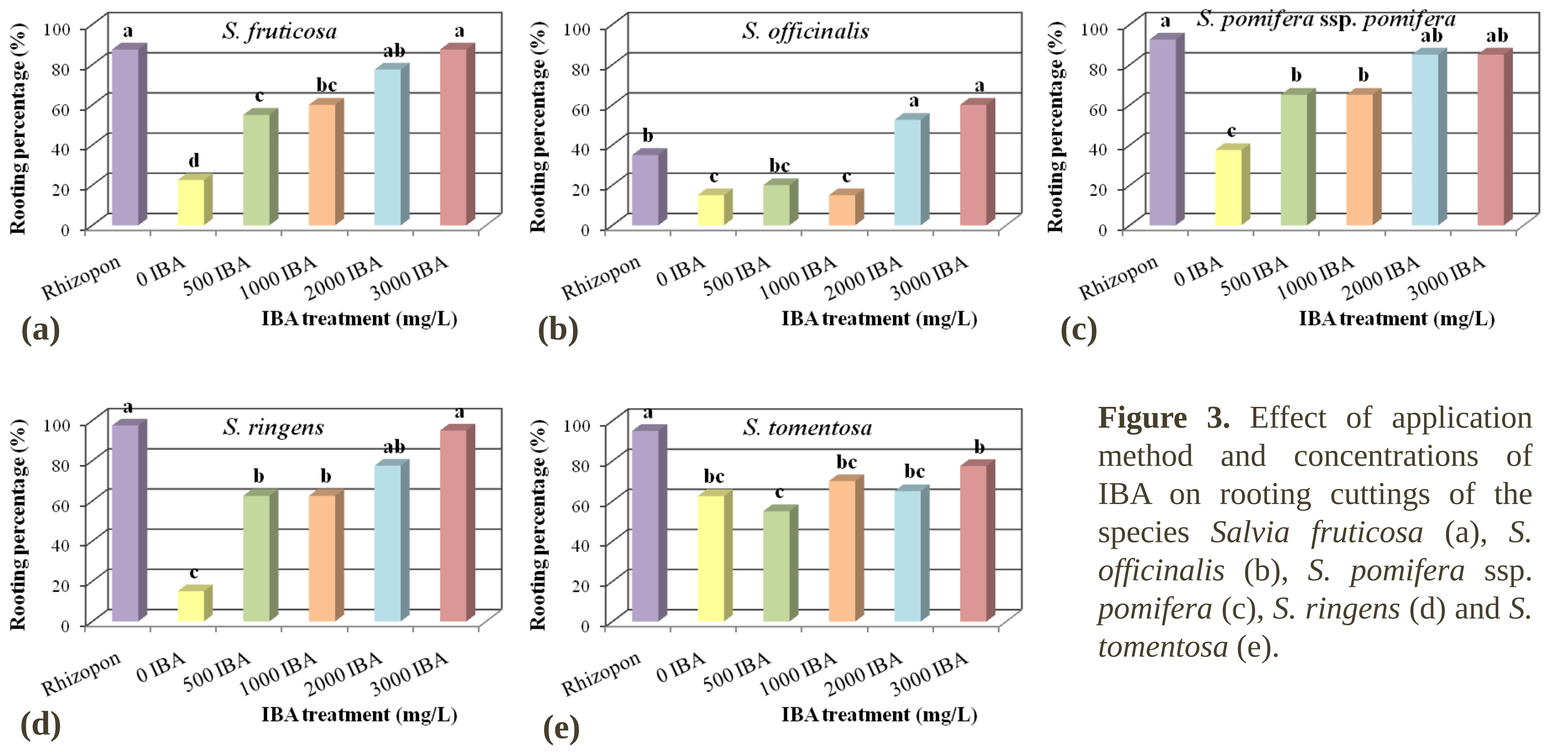

Figure 3. Effect of application method and concentrations of IBA on rooting cuttings of the species *Salvia fruticosa* (a), *S. officinalis* (b), *S. pomifera* ssp. *pomifera* (c), *S. ringens* (d) and *S. tomentosa* (e).

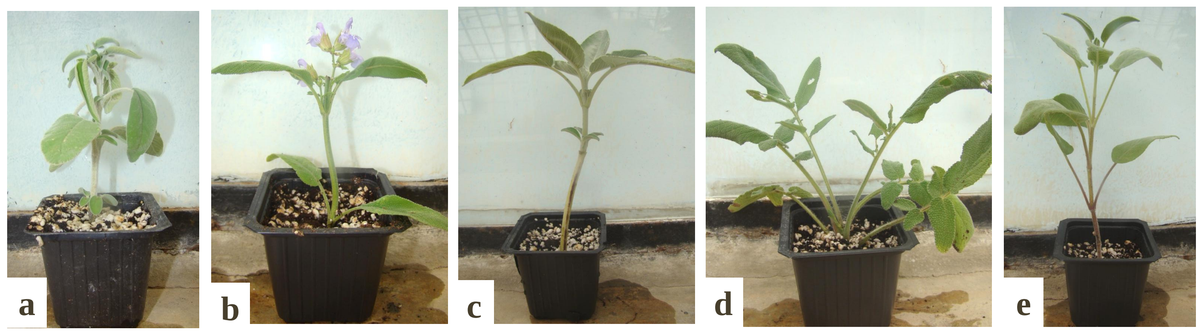

Figure 4. Rooted cuttings of the species *Salvia fruticosa* (a), *S. officinalis* (b, *S. pomifera* ssp. *pomifera* (c), *S. ringens* (d) and *S. tomentosa* (e).

Literature Cited

Paradiković, N., Zeljković, S., Tkalec, M., Vinković, T., Dervić, I. and Marić, M. 2013. Influence of rooting powder on propagation of sage (*Salvia officinalis* L.) and rosemary (*Rosmarinus officinalis* L.) with green cuttings. Poljoprivreda19: 10-15.

Kaçar, O., Azkan, N. and Çöplü, N. 2009. Effects of different rooting media and indole butyric acid on rooting of stem cuttings in sage (*Salvia officinalis* L. and *Salvia triloba* L.). Journal of Food, Agriculture and Environment 7: 349-352.

BGL 2019 Botanical Gardens & Landscapes

The First International Symposium on Botanical Gardens and Landscapes (BGL 2019)
December 2-4, 2019
Bangkok, Thailand

ISHS

* Project: SALVIA-BREED-GR. This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code:T1EDK-04923).

https://www.salvia-breed-gr.com/el/

European Union
European Regional Development Fund

EPAnEK 2014-2020
OPERATIONAL PROGRAMME
COMPETITIVENESS • ENTREPRENEURSHIP • INNOVATION

ΕΣΠΑ 2014-2020
Partnership Agreement 2014 - 2020