Comparative growth of rooted cuttings of five Mediterranean sage species (*Salvia* sp.)

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Abstract

In the present study, the effect of substrate type and fertilization on growing rooted cuttings of five wild species of sage found in Greece, *Salvia fruticosa*, *S. officinalis*, *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa* was examined, aiming to develop an effective nursery-cultivation method, for their introduction to the horticultural industry. Rooted cuttings were transplanted in various substrates of peat:perlite (1:1, 2:1 or 3:1, v/v) in plastic pots, and were fertilized once a month with 2 or 4 g L^{-1} water-soluble fertilizer (Nutrileaf 60, 20-20-20). Plants were maintained for four months (June to September 2019) in a greenhouse and their final height and axillary-shoot number and length were recorded. In *S. pomifera* ssp. *pomifera*, plants fertilized with 4 g L^{-1} were taller than those fertilized with 2 g/L^{-1}, regardless of substrate. High fertilization caused extreme elongation of the central shoot in *S. tomentosa* resulting to plant bending. In the other three species, there were no differences in central shoot length (plant height) among treatments. More axillary shoots were formed by plants fertilized with 4 g L^{-1} in *S. fruticosa* and *S. pomifera* ssp. *pomifera*, by plants cultivated in peat:perlite 3:1 in *S. ringens* and by all treatments, excepting cultivation in peat:perlite 1:1 and fertilization with 2 g/L, in *S. tomentosa*. Generally, there were no differences among treatments in axillary shoot length, excepting *S. tomentosa*, in which the longest axillary shoots were produced by plants in peat:perlite 2:1. Although all species grew satisfactorily regardless of the experimental treatments, fertilization with 2 g L^{-1} could be suggested to produce more compact plants.

Keywords: fertilization, native xerophytic ornamentals, substrate, peat, perlite

INTRODUCTION

There is a trend to introduce native plant species as ornamental plants or landscape plants for green spaces and green roofs in particular, especially in dry and hot climates (Papafotiou et al., 2013a,b; Toscano et al., 2014; Akoumianaki-Ioannidou et al., 2015; Tassoula et al., 2015; Zucchi et al., 2019; Bastos et al., 2020), as they are adapting well to local conditions and contribute to the preservation of biodiversity (Cervelli et al., 2012; Salisbury et al., 2020). Use of Mediterranean *Salvia* species in xeriscape landscaping could have many benefits, such as reduced water and cultivation requirements, high ornamental value (rich inflorescence and color diversity of flowers), as well as attraction of bees and other pollinators (Hung et al., 2015; Giovanetti et al., 2020). Simultaneously those species could be attractive pot plants or cut flowers.

There were a few reports found on propagation of Mediterranean salvia species (Erisen et al., 2020; Markovic et al., 2020), and a number on nursery growth of native Mediterranean xerophytes (Martini et al., 2017; Póvoa et al., 2019; Fascella et al., 2020), which showed that substrate type and fertilization can strongly affect growth and growth habit of these species.

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The five *Salvia* species of this study are fragrant bushes with high variability in flower color from white and blue to pink and violet, in March to August, depending on the species. Some are used as herbal tea with medicinal properties (Blamey & Grey-Wilson, 1993).

As part of a research program (SALVIA-BREED-GR, https://www.salvia-breed-gr.com/en/), which concerns breeding and promotion of sage species native in Greece for horticultural use, in the present study, the effect of substrate type and fertilization on growing rooted cuttings of *S. fruticosa*, *S. officinalis*, *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa,* was investigated, aiming to develop an efficient nursery-cultivation method.

MATERIALS AND METHODS

Rooted cuttings of *Salvia fruticosa*, *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa* (Figure 1), eight-weeks old, were transplanted end of May 2019 on various substrates of peat-perlite (1:1, 2:1 or 3:1, v/v), in plastic pots (1.3 L), and were fertilized once a month with 2 or 4 g L^{-1} water-soluble fertilizer (Nutrileaf 60, 20-20-20, Mg 0.0251 %, Cu 0.05%, B 0.02%, Fe 0.10%, Mn 0.05%, Zn 0.05%, Mo 0.001 %). Rooted cuttings of *S. officinalis* (Figure 1) were transplanted only on a peat-perlite 2:1 (v/v) substrate and were fertilized with 2 or 4 g L^{-1} fertilizer, due to lack of sufficient number of rooted cuttings, because of their low rooting percentage caused by insufficient lignification and the fact that they were all in blooming (Martini et al., 2020). Plants were maintained in a glasshouse and were initially watered once every 5 days. As plants grew, the water requirements increased throughout the summer season and at the end of summer, they were watered three times a week. Their growth was evaluated at the end of September 2019, recording central shoot length (from the level of the pot rim to the highest leaf), and axillary-shoot number and length. The completely randomized design and four repetitions of ten cuttings per treatment were used, the significance of the results was tested by three-ways analysis of variance (ANOVA) for the four species, excepting *S. officinalis*, as well as one- and two-way analysis of variance (ANOVA) for each species, while treatment means were compared by Student's *t* test at $p \leq 0.05$. Three-way ANOVA for all growth parameters showed significant interaction of the experimental factors (plant species, substrate, fertilization) so, data were analyzed separately for each species using two-way ANOVA.

Figure 1. Rooted cuttings used as plant material in the experiments. *Salvia fruticosa* (a), *S. officinalis* (b), *S. pomifera* ssp. *pomifera* (c), *S. ringens* (d) and *S. tomentosa* (e). Size bars = 5 cm.

RESULTS AND DISCUSSION

Central shoot length did not differ between treatments in *S. fruticosa* and *S. ringens* (Figure 2a; Figure 3a, b and d), neither in *S. officinalis* (17.4 and 14.8 cm for 2 and 4 g/L^{-1}, respectively). *S. officinalis* plants were the shortest of all species, because main shoot was not elongated due to the blooming of cuttings (Figure 2a; Figure 3). In *S. pomifera* ssp. *pomifera* plants fertilized with 4 g L^{-1} were taller than those fertilized with 2 g/L^{-1}, regardless of substrate (Figures 2a and 3c). *S. tomentosa* showed intense bending in greenhouse cultivation, and the highest elongation of all species. Highest elongation in this species was observed in peat:perlite 2:1 fertilized with 4 mg L^{-1} significantly different from peat:perlite 1:1 or 3:1 with the same fertilization and from peat:perlite 1:1 with 2 mg L^{-1} fertilization (Figure 2a), while increasing peat content in the substrate or fertilization intensified shoot bending (Figure 3e). Peat:perlite ratio and amount of fertilization has been shown in previous works as well, to affect strongly shoot elongation and plant shape inducing shoot bending, in a number of Mediterranean species (Papafotiou et al., 2000b; Akoumianaki - Ioannidou et al., 2016 and Martini et al., 2017).

Regarding axillary shoot number, in *S. fruticosa* and *S. pomifera* ssp. *pomifera*, more shoots were formed by plants fertilized with 4 g L^{-1} than with 2 g L^{-1}, in all substrates (Figure 2b; Figure 3a and c). In *S. ringens*, axillary shoot number was greater in plants transplanted in peat:perlite 3:1, irrespectively fertilization (Figures 2b and 3d), while in *S. tomentosa*, equal number of axillary shoots was produced in all treatments, excepting transplantation in peat:perlite 1:1 and fertilization with 2 g L^{-1} (Figures 2b and 3e). More axillary shoots per plant were also produced by *Teucrium capitatum* cuttings (Martini et al., 2017) or *Lavandula stoechas* seedlings (Papafotiou et al., 2000b), when they were fertilized with the highest or the most frequent dose. In *S. officinalis*, axillary shoot number was not affected by fertilization dose (3.8 axillary shoots for both fertilizations) (Figure 3b), similar to the results reported for *Euphorbia characias* (Papafotiou et al., 2000a). *S. tomentosa* developed most axillary shoots followed by *S. pomifera* ssp. *pomifera,* while *S. ringens* developed the fewest shoots (Figure 2b).

Axillary shoot length was similar in all treatments in most species, excepting *S. tomentosa*, in which length of axillary shoots was highest in plants transplanted in peat:perlite 2:1 (Figures 2c and Figure 3). In *S. officinalis*, axillary shoots of equal length (10.5-12.3 cm) were also produced in both fertilization doses (Figure 3b).

Conclusively, all species grew satisfactorily irrespectively of substrate and fertilization treatments, but fertilization with 2 g L^{-1} could be proposed in order to produce more compact plants, with less bending shoots, especially in case of *S. tomentosa*.

ACKNOWLEDGEMENTS

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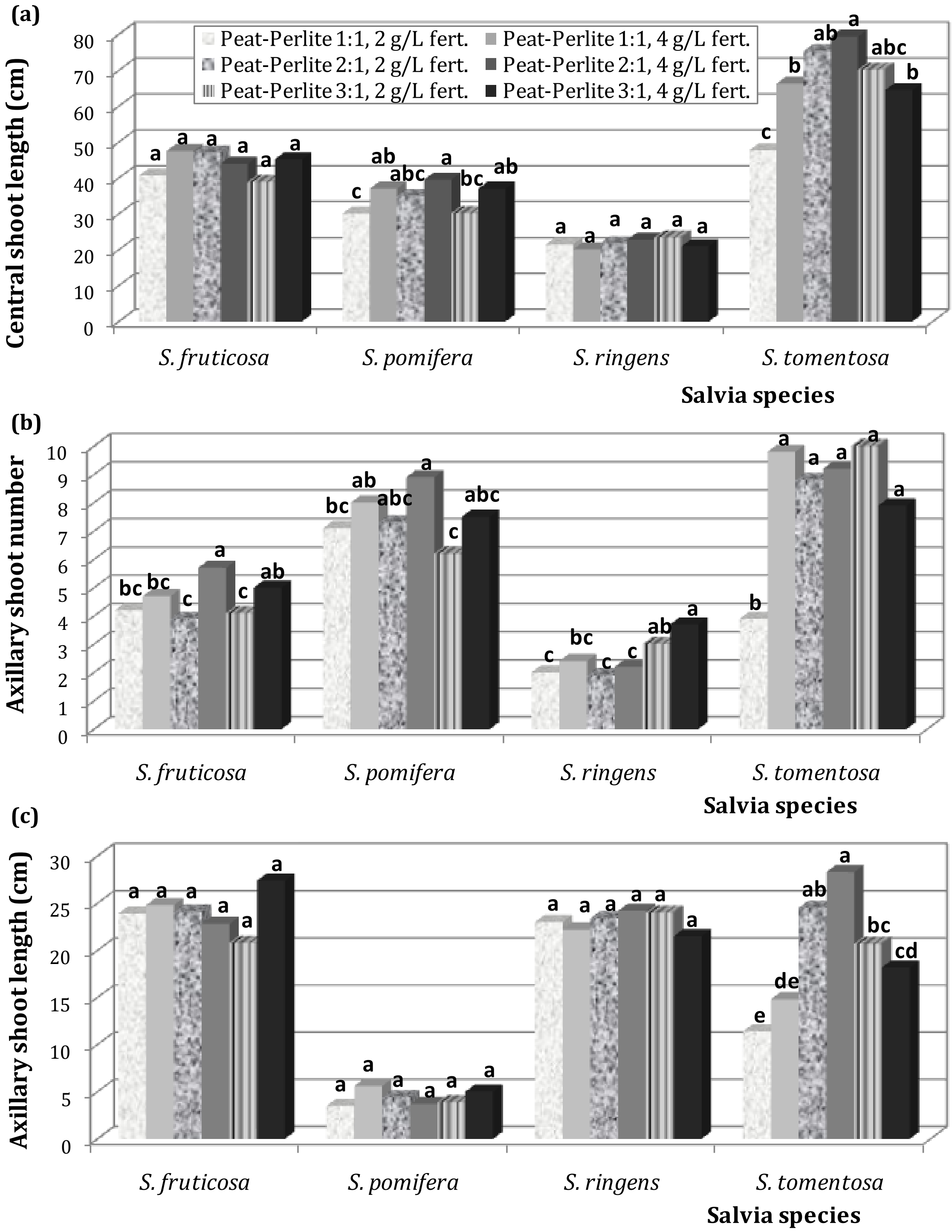

Figure 2. Effect of substrate type and amount of fertilization on (a) central shoot length, (b) axillary shoot number and (c) axillary shoot length, of rooted cuttings of five species of sage native in Greece three months after transplantation on the substrates shown. Mean values for each species followed by the same lower-case letter do not differ significantly at $P \leq 0.05$ by Student's t test.

Figure 3. Typical growth of rooted cuttings of the species *Salvia fruticosa* (a), *S. officinalis* (b), *S. pomifera* ssp. *pomifera* (c), *S. ringens* (d) and *S. tomentosa* (e), three months after transplantation on marked substrates (v/v): peat-perlite 1:1 (1 and 2), peat-perlite 2:1 (3 and 4) and peat-perlite 3:1 (5 and 6) and fertilizations: with 2 g L^{-1} (odd numbers) or 4 g L^{-1} (even numbers) fertilizer, respectively. Size bars = 10 cm.

Literature cited

Akoumianaki-Ioannidou, A., Martini, N.A., and Papafotiou, M. (2015). Rooting and establishment of *Limoniastrum monopetalum* (L.) Boiss stem-tip cuttings. Afr. J. Plant Sci. *10*(1), 23-31 e http://www.academicjournals.org/AJPS

Bastos, F.E.A., Grimaldi, F., Kretzschmar, A.A., and Rufato, L. (2020). Propagation of native plants with ornamental potential from Serra do Oratorio, Santa Catarina State, Brazil. Ornamental Hortic. *26*(2): 298-305 https://doi.org/10.1590/2447-536x.v26i2.2155

Blamey, M., and Grey-Wilson, C. (1993). Mediterranean wild flowers. Harper Collins Publishers, London. pp. 401-402.

Cervelli, C., Farina, E., Dalla Guda, C., Giovannini, A., Liotta, A., Paterniani, T., Burchi, G., Cacini, S., Antonetti, M., Zizzo, G., and Aprile, S. (2012). Development of new ornamental plants and germplasm selection in Mediterranean native species. Acta Hortic. *937*, 45-50 https://doi.org/10.17660/ActaHortic.2012.937.3

Erisen, H., Kurt-Gur, G., and Servi, H. (2020**).** In vitro propagation of *Salvia sclarea* **L**. by meta-Topolin, and assessment of genetic stability and secondary metabolite profiling of micropropagated plants. Industrial crops and products *157,* 112892 https://doi.org/10.1016/j.indcrop.2020.112892

Fascella, G., Rouphael, Y., Cirillo, C., Pannico, A., El-Nakhel, C. and De Pascale, S. (2020). Growth and quality response of potted ornamental shrubs under salt stress. Acta Hortic. 1296, 861-868 https://doi.org/10.17660/ActaHortic.2020.1296.109

Giovanetti, M., Giuliani, C., Boff, S., Fico, G., and Lupi, D. (2020). A botanic garden as a tool to combine public perception of nature and life-science investigations on native/exotic plants interactions with local pollinators. PLoS ONE *15*(2): e0228965 https://doi.org/10.1371/journal.pone.0228965

Hung, K.J., Ascher, J.S., Gibbs, J., *et al.* (2015). Effects of fragmentation on a distinctive coastal sage scrub bee fauna revealed through incidental captures by pitfall traps. *J Insect Conserv.* *19***,** 175–179 https://doi.org/10.1007/s10841-015-9763-8

Markovic, S.J., Brown, S.G., and Klett, J.E. (2020). Effects of growth substrate and container size on cutting production from Mojave sage stock plants. Hort Technology 30(4): 528-531. https://doi.org/10.21273/HORTTECH04620-20

Martini, A.N., Papafotiou, M., and Akoumianaki-Ioannidou, A. (2017). Vegetative propagation by stem cuttings and establishment of the Mediterranean aromatic and medicinal plant *Teucrium capitatum*. Acta Hortic. *1189*, 455-460 https://doi.org/10.17660/ActaHortic.2017.1189.90

Papafotiou, M., Tassoula, L., and Mellos, K. (2018). Construction and maintenance factors affecting most the growth of shrubby Mediterranean native plants on urban extensive green roofs. Acta Hortic. *1215*, 101-108 https://doi.org/10.17660/ActaHortic.2018.1215.18

Papafotiou, M., N., Pergialioti, E.A., Papanastasatos and L. Tassoula (2013a). Effect of Substrate Type and Depth and the Irrigation Frequency on Growth of Semiwoody Mediterranean species in Green Roofs. Acta Hortic., 990, 481-486.

Papafotiou, M., Pergialioti, N., Tassoula, L., Massas, I., and Kargas, G. (2013b). Growth of native aromatic xerophytes in an extensive Mediterranean green roof, as affected by substrate type and depth, and irrigation frequency. HortScience*, 48*(10): 1327-1333 https://doi.org/10.21273/HORTSCI.48.10.1327

Papafotiou, M., Garavelos, E., Antonopoulos, C., and Chronopoulos, J. (2000a). Studies on growth manipulation of Euphorbia characias L. Acta Hortic. *541*, 201-206 https://www.researchgate.net/deref/http%3A%2F%2Fdx.doi.org%2F10.17660%2FActaHortic.2000.541.28

Papafotiou, M., Garavelos, E., and Chronopoulos, J. (2000b). Effect of growing medium and fertilization on growth habit and colour of *Lavandula stoechas* L. Acta Hortic. *541*, 349-351

Póvoa, O., Vitorino, A., Mendes, J.P. and Farinha, N. (2019). Aromatic and medicinal plants vegetative propagation using reduced-cost nursery facilities. Acta Hortic. 1242, 905-910 https://doi.org/10.17660/ActaHortic.2019.1242.132

Salisbury, A., Al-Beidh, S., Armitage, J., *et al.* (2020). Enhancing gardens as habitats for soil-surface-active invertebrates: should we plant native or exotic species? Biodivers. Conserv. *29***,** 129-151 https://doi.org/10.1007/s10531-019-01874-w

Tassoula L., Papafotiou, M., Liakopoulos, G., and Kargas, G. (2015). Growth of native xerophytes *Convolvulus cneorum* L. on an extensive Mediterranean green roof under different substrate types and irrigation regimes. HortScience *50*(7):1118-1124 https://doi.org/10.21273/HORTSCI.50.7.1118

Toscano, S., Scuderi, D., Giuffrida, F., and Romano, D. (2014). Responses of Mediterranean ornamental shrubs to drought stress and recovery. Sci. Hortic. *178*, 145-153https://doi.org/10.1016/j.scienta.2014.08.014

Zucchi, M.R., Silva, M.W.da, Sibov, S.T., and Pires, L.L. (2019). Ornamental and landscape potential of a bromeliad native to the Cerrado. Ornam. Hortic. *25*(4), 425-433 http://dx.doi.org/10.1590/2447-536x.v25i4.2003

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INTRODUCTION

- Native plant species are introduced as ornamental/landscape plants in dry and hot climates while substrate type and fertilization can strongly affect their growth and their growth habit.
- The five *Salvia* species of this study are fragrant bushes with high variability in flower color. Some are used as herbal tea with medicinal properties .
- The effect of substrate type and fertilization on growing rooted cuttings of *Salvia fruticosa*, *S. officinalis*, *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa,* was investigated, aiming to develop an efficient nursery-cultivation method.

Figure 1. Rooted cuttings used as plant material in the experiments. *Salvia fruticosa* (a), *S. officinalis* (b), *S. pomifera* ssp. *pomifera* (c), *S. ringens* (d) and *S. tomentosa* (e). Size bars = 5 cm**.**

MATERIALS AND METHODS

- Rooted cuttings were transplanted on various substrates of peat-perlite (1:1, 2:1 or 3:1, v/v), in plastic pots (1.3 L), fertilized once a month with 2 or 4 g L^{-1} water-soluble complete fertilizer (Nutrileaf 60, 20-20-20).
- Plants were maintained (for 4 months) in a glasshouse and were initially watered once every 5 days and at the end of summer, they were watered three times a week.
- Plants' growth was evaluated at the end of the experiment, recording central shoot length, axillary-shoot number and length.

ACKNOWLEDGEMENTS

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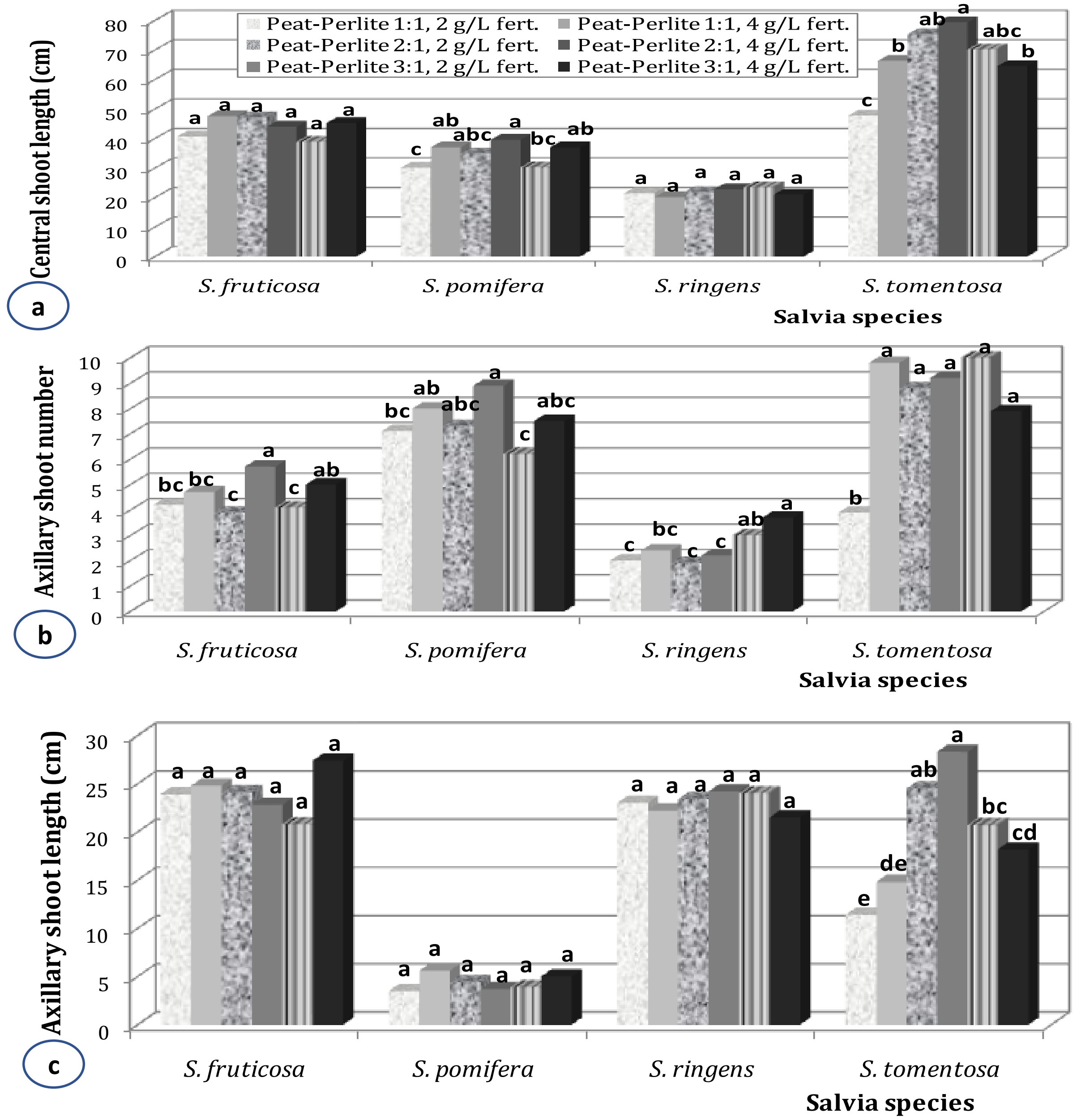

Figure 2. Effect of substrate type and amount of fertilization on (a) central shoot length, (b) axillary shoot number and (c) axillary shoot length, of rooted cuttings of five species of sage native in Greece three months after transplantation on the substrates shown. Mean values for each species followed by the same lower-case letter do not differ significantly at $P \leq 0.05$ by Student's *t* test. In all plant species' growth parameters $F_{interaction}^{NS}$; in (a) $F_{substrate}^{NS}$; $F_{fertilization}^{NS}$, apart from *S. officinalis*, *S. pomifera* and *S. tomentosa* where $F_{fertilization}^{*}$ at $P \leq 0.05$; in (b) $F_{substrate}^{*}$ at $P \leq 0.05$, apart from *S. fruticosa* where $F_{substrate}^{NS}$; in (c) $F_{substrate}^{NS}$, apart from *S. ringens and S. tomentosa* where $F_{substrate}^{*}$ at $P \leq 0.05$.

CONCLUSIONS

All species grew satisfactorily irrespectively of substrate and fertilization treatments, but fertilization with 2 g L^{-1} could be proposed in order to produce more compact plants, with less bending shoots, especially in case of *S. tomentosa*.

RESULTS AND DISCUSSION

- Central shoot length did not differ between treatments in *S. fruticosa* and *S. ringens* (Figure 2a; Figure 3a, b and d), neither in *S. officinalis* the shortest of all species (17.4 and 14.8 cm for 2 and 4 g/L^{-1}, respectively) (Figure 2a; Figure 3). In *S. pomifera* ssp. *pomifera* plants fertilized with 4 g L^{-1} were taller than those fertilized with 2 g/L^{-1}, regardless of substrate (Figures 2a and 3c). Highest elongation in *S. tomentosa* was observed in peat:perlite 2:1 fertilized with 4 mg L^{-1} (Figure 2a and Figure 3e).
- In *S. fruticosa* and *S. pomifera* ssp. *pomifera*, more shoots were formed by plants fertilized with 4 g L^{-1} than with 2 g L^{-1}, in all substrates (Figure 2b; Figure 3a and c), in *S. ringens* in peat-perlite 3:1, irrespectively fertilization (Figures 2b and 3d), while in *S. tomentosa*, equal number of axillary shoots was produced in all treatments, excepting transplantation in peat-perlite 1:1 and fertilization with 2 g L^{-1} (Figures 2b and 3e). In *S. officinalis*, axillary shoot number was not affected by fertilization dose (3.8 axillary shoots for both fertilizations) (Figure 3b). *S. tomentosa* developed most axillary shoots followed by *S. pomifera* ssp. *pomifera,* while *S. ringens* developed the fewest shoots (Figure 2b).
- Axillary shoot length was similar in all treatments in most species, excepting *S. tomentosa*, in which length of axillary shoots was highest in plants transplanted in peat-perlite 2:1 (Figures 2c and Figure 3). In *S. officinalis*, axillary shoots of equal length (10.5-12.3 cm) were also produced in both fertilization doses (Figure 3b).

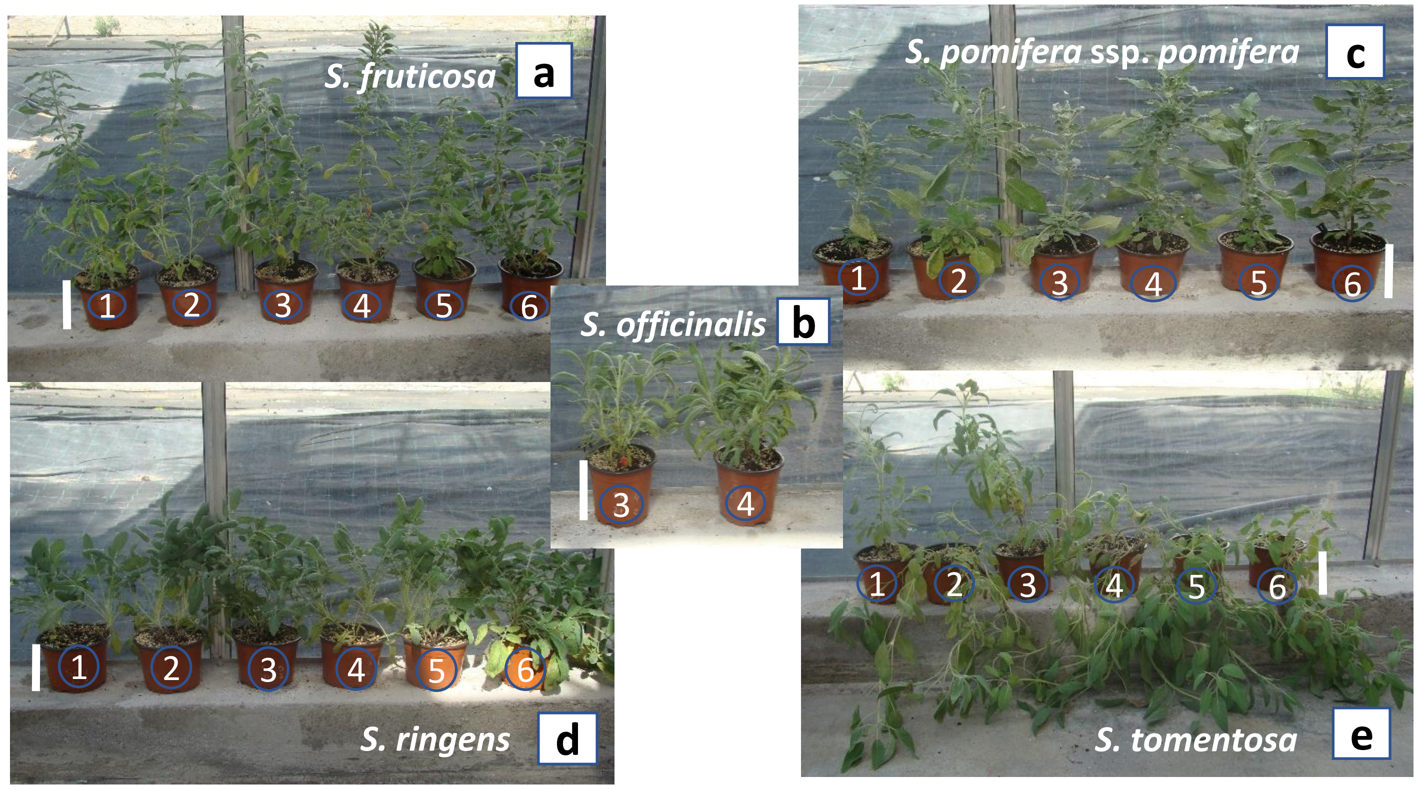

Figure 3. Typical growth of rooted cuttings of the species *Salvia fruticosa* (a), *S. officinalis* (b), *S. pomifera* ssp. *pomifera* (c), *S. ringens* (d) and *S. tomentosa* (e), three months after transplantation on marked substrates (v/v): peat-perlite 1:1 (1 and 2), peat-perlite 2:1 (3 and 4) and peat-perlite 3:1 (5 and 6) and fertilizations: with 2 or 4 g L^{-1} fertilizer, respectively. Size bars = 10 cm.