Effect of cutting type and indole butyric acid on propagation of *Salvia fruticosa* with cuttings

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Abstract

In the present study, the effect of cutting origin and indole-3-butyric acid (IBA) concentration and application method on rooting shoot cuttings of *Salvia fruticosa* Mill. (f. *Lamiaceae*), Greek sage, was investigated. The aim was to develop an effective propagation method that could enhance the potential use of the plant in the floriculture industry. Terminal and basal cuttings were collected from annual shoots of wild or greenhouse mother plants in early May. They were treated either with dusting powder for soft-wood cuttings Rhizopon (0.5% w/w IBA) or their bases were dipped for 1 min in solution IBA (50% ethanol) at five different concentrations, i.e., 0 (control), 500, 1500, 3000 and 4500 mg L^{-1}. Cuttings 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. Regarding cuttings derived from greenhouse mother plants, terminal ones rooted at higher percentage (80%) than the basal (60%), both after treatment with IBA powder, and the highest fresh and dry shoot weight and fresh root weight values were obtained after treatment with IBA powder, too. To the contrary, cuttings from wild mother plants rooted at highest percentage (80%) when basal and treated with 4500 mg L^{-1} IBA, while the highest rooting response for terminal cuttings was 55%. The use of IBA powder resulted to the highest fresh and dry weight of above-ground plant part in basal cuttings, but it reduced their rooting to 50%. As regards time needed for rooting, cuttings from greenhouse plants reached their highest rooting percentage faster (in two weeks) than cuttings from wild plants, which required four weeks. Therefore, an effective method for commercial propagation of *Salvia fruticosa* that would enhance use of desirable clones in xeriscaping, could involve terminal cuttings obtained from greenhouse grown mother plants and treated with IBA powder for soft-wood cuttings.

Keywords: terminal cuttings, basal cuttings, rooting, native xerophytic ornamentals, Greek sage

INTRODUCTION

Xeriscaping is a landscaping approach using small amounts of water maintaining a traditional look and using species selected for their water efficiency (Welsh, 2000; Ozyavuz and Ozyavuz, 2012). Mediterranean native *Salvia* species are ideal for xeriscaping because they require few cultivation practices and are drought tolerant. Furthermore, they are characterized by rich flowering and colour diversity of flowers traits of high ornamental value.

S*alvia fruticosa* Mill., Greek sage, ("faskomelo" in Greek, syn. *Salvia cypria* Unger & Kotschy), shows high adaptability to drought. It has white felted shoots, and its leaves are simple, having sometimes 1-2 pairs of small lobes below the main one. It is up to 1.20 m in height and its flowers are characterized high colour variability, from lilac to pink or even sometimes white, from March to June. *S. fruticosa* has widespread usage because of its aromatic leaves that are used for flavoring and for herbal tea (Blamey and Grey-Wilson, 1993). It is endemic to the Mediterranean basin, and it can be found in a wider zone from Sicily to Israel (Thanos and Doussi, 1995), from the sea level up to an altitude of 1500 m. In Greece it occurs in most of the littoral areas of the mainland, as well as on the Aegean and Ionian islands,

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forming extended populations in the Mediterranean ecosystem (Karousou and Kokkini, 1997). It has also a folk reputation as a hypoglycemic agent (Perfumi et al., 1991). Medicinal and aromatic herbs are characterized by low seed viability and low germination capacity because of the lack of seed selection and processing programs (Nicola et al., 2005). Vegetative propagation of medicinal and aromatic plants is a fast, well-established method being simpler in comparison with other asexual methods like micropropagation, grafting or budding (Paradiković et al., 2013). It is an attractive, fast method for plant production industry leading to a high uniformity of the plant material with the mother plants (Hartmann et al., 1997; Ford et al., 2002).

Exogenous application of auxin accelerates both the rates of rooting and increases final rooting percentage and the number of produced roots in leafy cuttings (de Klerk et al., 1999; Damtew Zigene and Mengesha Kassahun, 2016). There are some preliminary reports on rooting cuttings of *S. fruticosa* (Sağlam et al., 2014; Martini et al., 2020); thorough studies are required to determine the appropriate rooting hormone treatment and the cutting type.

As part of a research program (SALVIA-BREED-GR), aiming to the improvement and promotion of Greek sage species for ornamental use, in the present study, the effect of the mother plant, cutting type and indole-3-butyric acid (IBA) concentration and application method on rooting shoot cuttings of *Salvia fruticosa* was investigated, so that an effective propagation method could be established.

MATERIALS AND METHODS

The research took place in a glasshouse (37°58'53.94"N, 23°42'25.01"E), in Athens, Greece, from early May to July 2020. Cuttings were collected both from wild *S. fruticosa* plants in mount Hymettus and two-year-old glasshouse grown plants. Two types of cuttings were used, i.e., either the 10-12 cm terminal part of the annual stem (terminal cutting) or the just below this 10-12 cm stem part (basal cutting) (Figure 1).

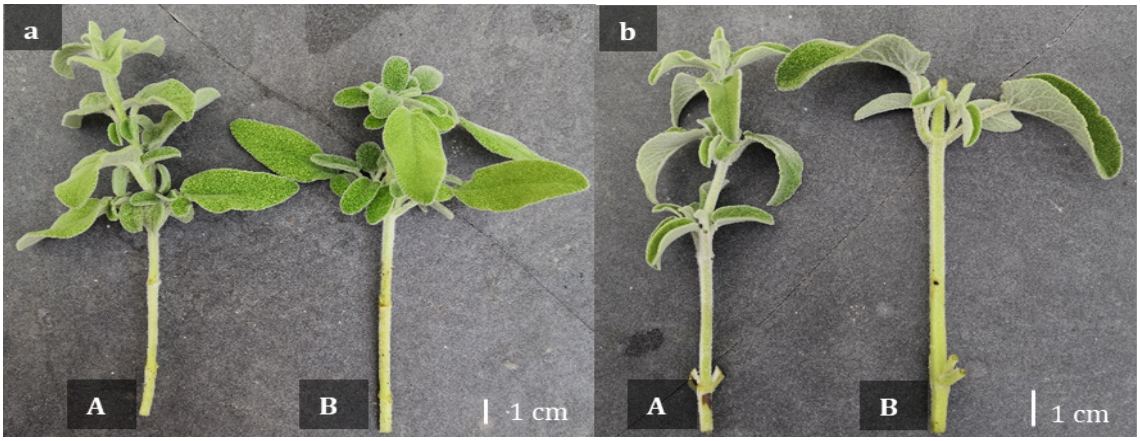

Figure 1. Typical terminal (A) and basal (B) cuttings, collected in May 2020, from greenhouse grown (a) and native (b) mother plants of *Salvia fruticosa.*

The cuttings were treated either with dusting powder for soft-wood cuttings 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 1500 or 3000 or 4500 mg L^{-1} and were placed for rooting on peat-perlite 1:1 (v/v), in 7 cm square, plastic pots, in a mist for 2 weeks. Then, they remained on the greenhouse bench in a semi-shaded location for another 4 weeks and were irrigated by hand.

On the 2nd and 4th week after the placement of cuttings in the rooting medium, the inspection of root formation took place by pulling the cutting, and rooting data collected. After 6 weeks, rooted cuttings (plants) were taken out of the pots and the roots of each plant were washed under tap water and dried with filter paper. Rooting percentage, shoot and root fresh

and dry weight were recorded. Dry weight was recorded after the above ground plant part and the rooting system were dried at 65°C for 5 days.

A completely randomized design and four replications of ten cuttings per treatment were used, the significance of the results was tested by three-, two- and one-way analysis of variance (ANOVA) and treatment means were compared by Tukey test at p≤0.05. The data of percentages were statistically analysed after arcsine transformation.

RESULTS AND DISCUSSION

Three-way ANOVA of cuttings' rooting percentages showed significant interactions the main experimental factors, i.e., mother-plant type (greenhouse grown or wild), cutting type (terminal or basal) and IBA treatment (three-way ANOVA results not presented). Similarly, significant interaction showed the two-way ANOVA of cuttings' rooting percentages concerning IBA treatment and cutting type (two-way ANOVA results not presented). Therefore, rooting data were analysed separately for each mother-plant type and cutting type as for the effect of IBA treatment by one-way ANOVA. For cuttings derived from greenhouse plants higher rooting percentages were occurred after treatment with dusting powder Rhizopon, both for terminal and basal cuttings (80 and 60%, respectively, Figures 2a, b and 3a), in agreement with a previous report (Martini et al., 2020).

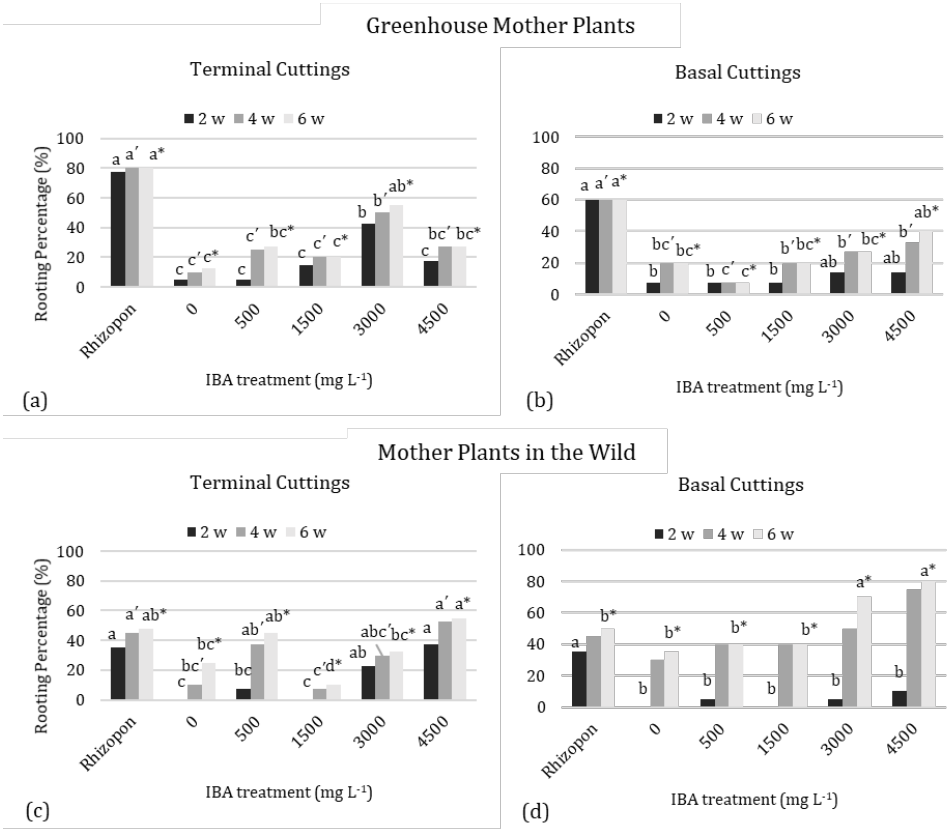

Figure 2. Effect of IBA concentration and application method on rooting of *Salvia fruticosa* cuttings derived from either greenhouse plants (a, b), or wild plants (c, d). Letters followed by (') or (*) represent 4- and 6-weeks statistic respectively, while clear letters represent 2-weeks statistic. Data analysed by one-way ANOVA for each mother-plant origin, i.e., greenhouse and wild plants and at each data collecting date, i.e., 2, 4 and 6 weeks (w). Means of each mother plant origin and data recording date, followed by the same letter are not significantly different according to Tukey test at p≤0.05.

Figure 3. Rooted terminal cuttings treated with IBA methods presented and typical rooting system of them; cuttings derived either from greenhouse (a) or wild (b) *Salvia fruticosa* plants. D: dusting powder for soft-wood cuttings Rhizopon (0.5% w/w IBA); IBA solutions at concentration (mg L^{-1}) presented.

As regards cuttings derived from plants in the wild, rooting percentage was highest (80%) for basal cuttings treated with 4500 mg L^{-1} IBA (Figure 2d). The highest rooting percentage of terminal cuttings was 55% and was achieved after treatment with 4500 mg L^{-1} IBA, too (Figure 2c). The opposite effect of cutting type on rooting comparing green-house and wild-origin cuttings could be attributed to the higher degree of lignification of basal cuttings derived from plants grown in the wild.

In a previous study (Martini et al., 2020) Rhizopon, as well as IBA solutions, induced higher rooting percentages on terminal cuttings from plants in the wild compared to the results of the present study. This could be attributed to that a) cuttings in the present work were lignified at a higher degree giving that they were collected one month later, and b) they were in blooming, in agreement with similar effects concerning rooting of *S. officinalis* cuttings (Capecka, 2012). The degree of rooting of stem cuttings and period necessary for root formation depends on the date of cutting collection and the development stage of mother plant (Klein et al., 2000), in relation to changes in the endogenous plant growth regulators or carbohydrate conditions of cuttings and the environmental conditions (Elgimabi, 2008).

Regarding the required time for rooting, cuttings from greenhouse mother plants rooted faster, reaching the maximum of their rooting percentage at only two weeks for most treatments, compared to cuttings collected from mother plants in the wild, which reached their maximum rooting percentage after 4-6 weeks (Figure 2a-d). The effect of faster rooting was reflected in the fresh and dry weight of the above ground part of plantlets, while for most treatments there were no differences in fresh and dry weight of roots (Table 1). Three-way ANOVA showed significant interactions. Two-way ANOVA separately for greenhouse and wild mother-plant origin of cuttings showed in most cases interaction between IBA treatment and cutting type; however, in cuttings from wild plants, IBA treatment affected the fresh and dry weight of the above ground part, and Rhizopon resulted in higher weight values in basal

cuttings (Table 1).

Table 1. Effect of IBA concentration and application method on fresh and dry weight of above ground part and roots of *Salvia fruticosa* plantlets obtained from stem cuttings derived from either greenhouse plants or plants in the wild. Data was collected after 6 weeks from cutting placement for rooting.

Cutting type	IBA treatment (mg L^{-1})	Fresh weight (g)		Dry weight (g)	
		Shoots	Roots	Shoots	Roots
Mother plants from the greenhouse					
Terminal	Rhizopon	4.82 a	1.62	1.04 a	0.25 b
	0	2.73 bc	1.62	0.71 bcd	0.33 b
	500	2.77 bc	1.42	0.66 cd	0.25 b
	1500	3.00 bc	1.17	0.83 bc	0.30 b
	3000	2.88 bc	1.27	0.79 bc	0.28 b
	4500	3.48 b	1.68	0.36 d	0.93 a
Basal	Rhizopon	3.93 ab	1.80	1.06 a	0.36 b
	0	2.65 bc	1.42	0.74 bc	0.31 b
	500	1.60 c	0.60	0.60 cd	0.29 b
	1500	3.29 bc	1.41	1.03 ab	0.33 b
	3000	2.43 bc	1.17	0.73 bc	0.40 b
	4500	2.17 bc	1.07	0.65 cd	0.29 b
Mother plants from the wild					
Terminal	Rhizopon	2.95	1.50 ab	0.77	0.29 abc
	0	2.31	1.13 b	0.69	0.19 c
	500	2.48	1.38 ab	0.67	0.30 abc
	1.500	1.94	0.98 b	0.63	0.27 abc
	3.000	3.21	1.44 ab	0.83	0.26 abc
	4.500	2.81	1.93 ab	0.82	0.35 a
Basal	Rhizopon	4.44	2.18 a	0.95	0.36 a
	0	1.80	1.18 ab	0.42	0.27 abc
	500	1.98	1.29 ab	0.57	0.20 bc
	1500	2.70	1.54 ab	0.64	0.25 abc
	3000	1.92	1.59 ab	0.58	0.33 ab
	4500	2.27	1.72 ab	0.62	0.29 abc
Significance of two-way ANOVA					
Mother plants from the greenhouse					
F_{IBA}		-	*	-	-
$F_{cutting}$		-	-	-	-
$F_{IBA \times cutting}$		*	-	*	*
Mother plants from the wild					
F_{IBA}		**	-	**	-
$F_{cutting}$		**	-	-	-
$F_{IBA \times cutting}$		-	**	-	**

Mean separation in columns by Tukey test at p≤0.05; *, **: significant at p≤0.05, p≤0.01, respectively.

In previous studies on other *Salvia* species (*S. fruticosa* and *S. officinalis*), the use of rooting auxin-products even at low concentrations enhanced out-of-season rooting of cuttings having a positive effect on root number and length (Nicola et al., 2003, 2005), plant height, number of leaves, fresh and dry weight of plants (Paradiković et al., 2013; Sağlam et al., 2014). In the present study, auxin at low concentrations did not stimulate better rooting of cuttings and plantlet growth.

Conclusively, mother plant, cutting position and auxin treatment affected propagation ability of *S. fruticosa* by stem cuttings. The most effective method for asexual propagation of

Salvia fruticosa was the use of terminal cuttings obtained from greenhouse-grown mother plants and treated with dusting powder Rhizopon (0.5% w/w IBA), resulting in high (80%) and fast (two week) rooting.

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Effect of cutting type and indole butyric acid on propagation of *Salvia fruticosa* with cuttings

Salvia-Breed-GR

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Introduction

Mediterranean native *Salvia* species are ideal for xeriscaping

Salvia fruticosa:

- high adaptability to drought
- Endemic to the Mediterranean basin
- Strongly aromatic up to 1.00 m in height
- Very attractive during flowering, bearing pink inflorescences

As part of a research program (SALVIA-BREED-GR), aiming to the improvement and promotion of Greek sage species for ornamental use, in the present study, rooting of stem cuttings of *Salvia fruticosa* was investigated, as affected by

mother plant,
cutting type,
application method and concentration of indole-3-butyric acid (IBA)

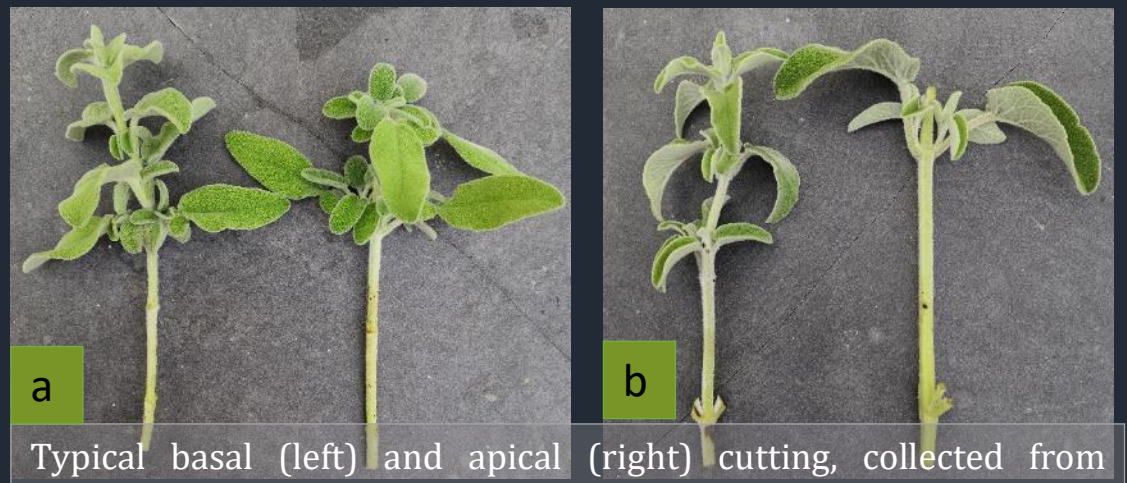
Materials and Methods

- Glasshouse
- Early May to July of 2020
- Apical and basal cuttings, 10-12 cm long
- Cuttings were collected both from:
  -native plants, in mount Hymettus
  -glasshouse grown mother plants, two years old.

Treatments
dusting powder for soft wood cuttings Rhizopon (0.5% w/w IBA)
cutting base dipped for 1 min in a
solution IBA 0/500/1500/3000/4500 mg L^{-1}

Rooting on peat-perlite 1:1 (v/v)
plastic pots
in a mist for 2 weeks.

They remained on the greenhouse bench in a semi-shaded location for another 4 weeks and were irrigated by hand.



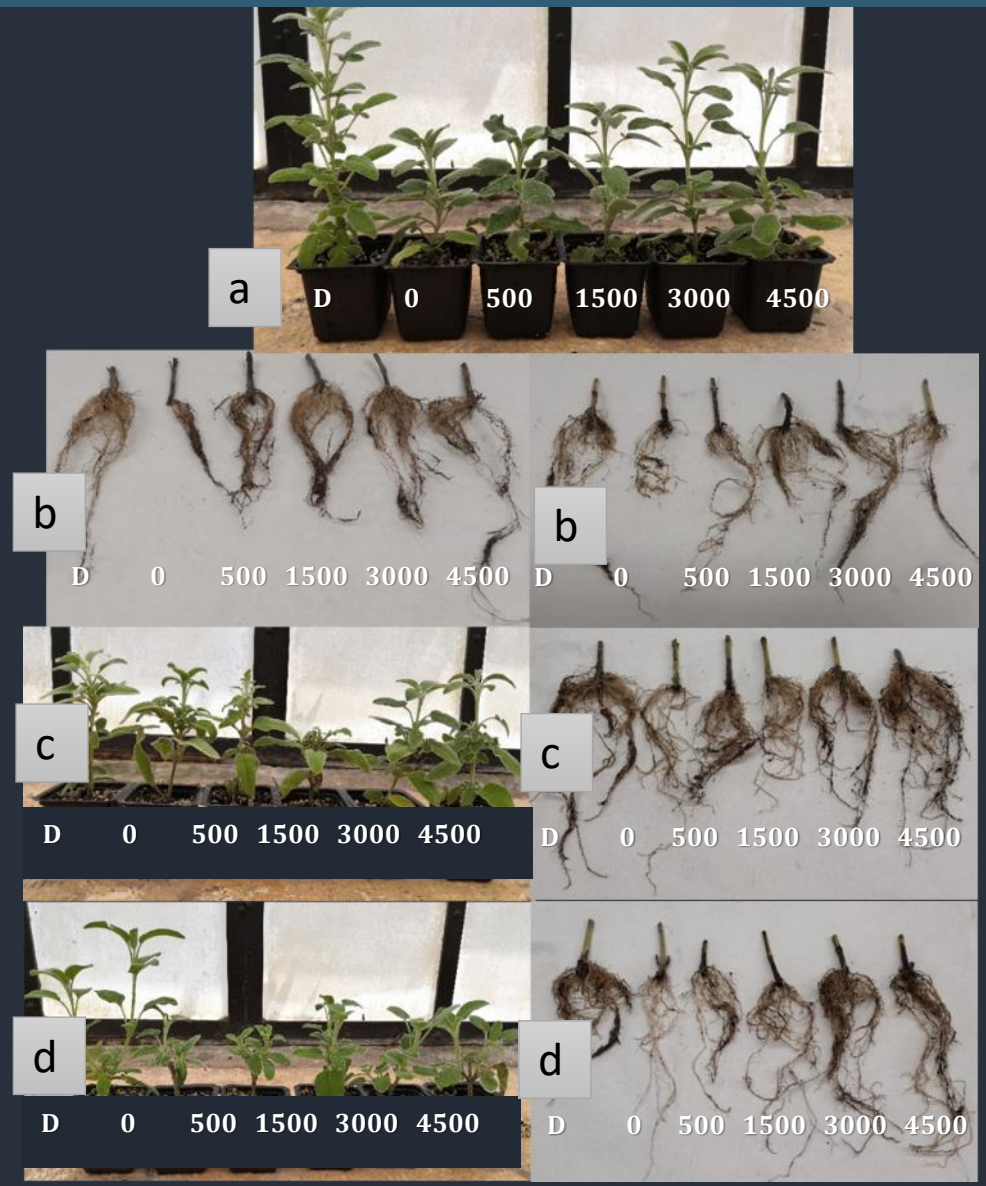
Typical basal (left) and apical (right) cutting, collected from greenhouse grown (a) and native (b) mother plants of *S. fruticosa*

Data collected on the 2nd , 4th and 6th week

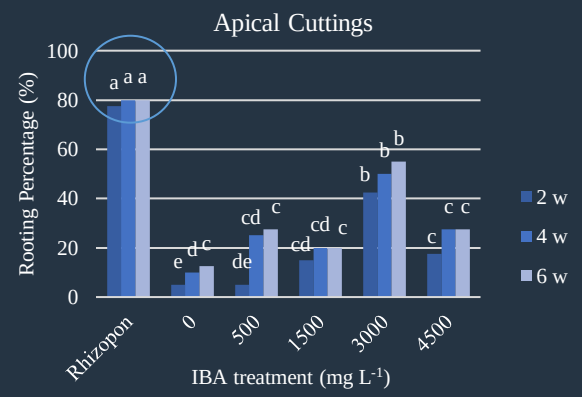
6th week:
rooting percentage,
stem and root fresh and dry weight were recorded.

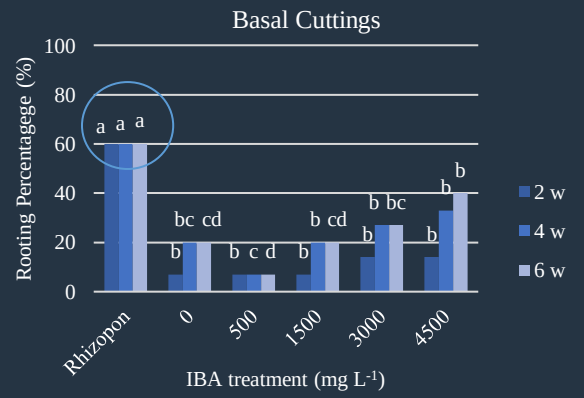
The completely randomized design and four repetitions of ten cuttings per treatment were used, the significance of the results was tested by one-way analysis of variance (ANOVA) and treatment means were compared by Student's t test at $p \leq 0.05$.

Results and Discussion



Rooted cuttings and typical rooting system of them, derived from greenhouse grown (a and b) and native (c and d) mother plants of *Salvia fruticosa* and treated with marked IBA concentration (mg L^{-1}), for apical and basal cuttings, respectively. D: dusting powder Rhizopon

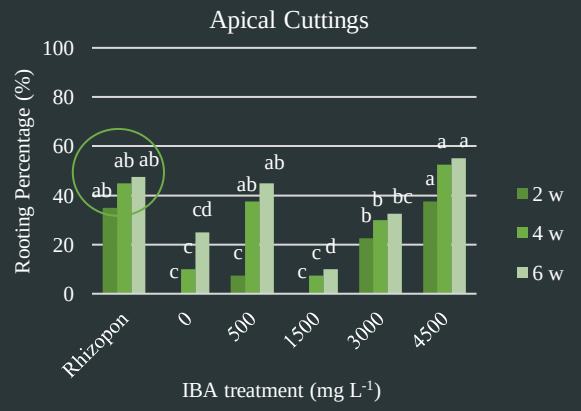


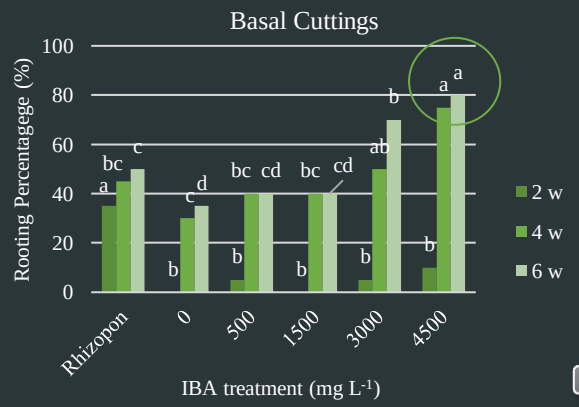


Greenhouse plants

Effect of application method and concentration of IBA on fresh and dry weight of above ground plant part and roots of *Salvia fruticosa* cuttings from either greenhouse or wild mother plants

Cutting	IBA treatment (mg L^{-1})	Fresh weight (g) Stems	Fresh weight (g) Roots	Dry weight (g) Stems	Dry weight (g) Roots
		Plants from the greenhouse			
Apical	Rhizopon	4.82 a	1.62 ab	1.04 a	0.25 e
	0	2.73 cd	1.63 ab	0.71 c	0.33 cde
	500	2.77 cd	1.42 ab	0.66 c	0.25 de
	1500	3.00 bcd	1.17 b	0.83 bc	0.30 cde
	3000	2.88 cd	1.27 b	0.79 c	0.28 de
	4500	3.48 bc	1.68 a	0.36 d	0.93 b
Basal	Rhizopon	3.93 b	1.80 a	1.06 a	0.36 cd
	0	2.65 cd	1.42 ab	0.74 c	0.31 cde
	500	-			
	1500	3.29 bcd	1.41 ab	1.03 ab	1.76 a
	3000	2.43 d	1.17 b	0.73 c	0.40 c
	4500	2.17 d	1.07 b	0.65 c	0.29 cde
		Plants from the wild			
Apical	Rhizopon	2.95 bc	1.50 bcd	0.77 ab	0.29 abc
	0	2.31 def	1.13 d	0.69 bc	0.19 d
	500	2.48 cde	1.38 cd	0.67 bc	0.30 abc
	1.500	1.94 ef	0.98 cd	0.63 bcd	0.27 abcd
	3.000	3.21 b	1.44 cd	0.83 ab	0.26 cd
	4.500	2.81 bcd	1.93 ab	0.82 ab	0.35 ab
Basal	Rhizopon	4.44 a	2.18 a	0.95 a	0.36 a
	0	1.80 f	1.18 dc	0.42 d	0.27 abcd
	500	1.98 ef	1.29 cd	0.57 cd	0.20 d
	1500	2.70 bcde	1.54 bcd	0.64 bcd	0.25 cd
	3000	1.92 f	1.59 bcd	0.58 cd	0.33 ab
	4500	2.27 def	1.72 abc	0.62 cd	0.29 abc





Plants in the wild

Conclusions

- mother plant
  - cutting position
    - hormone treatment

- Affected propagation ability of *S. fruticosa*

- Apical cuttings
- Treatment with dusting powder
- Quick method to propagate

- Basal cuttings
- Immersion
  In a 4500 mg L^{-1} IBA (1 min)

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