Scientia Horticulturae 337 (2024) 113452

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Research Paper

Influence of water spraying intervals and indole-3-butyric acid concentrations on *Salvia* rooted cuttings quality in a closed aeroponics system

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ARTICLE INFO

Keywords:
Clonal propagation
Ornamentals
Root development
Sage
Soilless cultivation

ABSTRACT

Aeroponics, a soilless cultivation system where plants grow suspended in air and nutrient solution is sprayed directly onto the root system, can potentially optimize clonal stem cutting propagation. Here, we evaluated the effects of water spray intervals and the Indole-3-butyric acid (IBA) treatment on adventitious root development and the quality of ornamental sage cultivar cuttings. The investigation focused on three factors: (a) spray intervals of 5 (I_5) and 10 min (I_{10}); (b) IBA of 0 g L^{-1} (C_0 - control) and with a concentration of 1 g L^{-1} (C_1); and (c) sage cultivars 'Farina Silver Blue' and 'La Siesta'. A significant interaction was found between spray interval and IBA concentration in developing rooted cuttings. Specifically, applying 1 g L^{-1} IBA with the longer spray interval of I_{10} positively influenced the percentage of cuttings with first roots at seven days post-cutting, the proportion of rooted cuttings, and the number of roots per cutting at 21 days post-cutting. The I_{10} spray interval yielded higher adventitious root quality than I_5. No significant differences were observed between IBA concentrations regarding root length, root area, or the number of tips, forks, and crossings, whereas untreated cuttings exhibited greater root diameter and volume. Statistical analysis further indicated that combining 1 g L^{-1} IBA with the I_{10} spray interval resulted in significantly higher root biomass yield. These findings suggest the potential for sustainable cutting production. Enriching aeroponics with these elements enables healthy, high-quality plant cultivation without using non-renewable resources. Aeroponics may also serve as a foundational technology for emerging systems like vertical farming.

1. Introduction

Salvia (sage) is the largest genus of the Lamiaceae family (Napoli et al., 2020), with over 960 herbaceous and woody species (Walker et al., 2004). Sage is cultivated for both ornamental and medicinal reasons, as they possess high-quality essential oils in all aboveground parts, especially leaves and flowers (De Mastro et al., 2021). Morphologically, sages have paired, simple, or pinnately lobed leaves and 2-lipped flowers in whorls, forming simple or branched spikes or racemes.

Salvia 'Farina Silver Blue' and 'La Siesta' are relevant ornamental cultivars in Mediterranean nurseries (Cervelli et al., 2011). The first, also known as mealy sage, is an upright perennial grown annually, with lance-shaped silvery green foliage, hairy on the reverse, and slender racemes of small light blue-lipped flowers in summer and autumn. *Salvia* 'La Siesta' has paired, simple, or pinnately lobed, often aromatic leaves and 2-lipped flowers in whorls, forming simple or branched racemes. Because of their essential oils, both cultivars exert numerous antioxidant, anti-inflammatory, cytotoxic, and antibacterial properties (Fraternale et al., 2012; Afonso et al., 2019; Carlini et al., 2022). Recent studies have shown that both cultivar's flowers are edible and possess relevant nutritional properties (Marchioni et al., 2023).

Modern challenges in agriculture have developed cultivation systems to increase yield and quality with more sustainable resource management (Toscano et al., 2023).

One such system is soilless culture systems (SCS), which allows a more rational use of nutrients and spaces without being dependent on soil-related issues (Santamaria et al., 2021; Gruda et al., 2023).

Botanists used the first primitive devices of aeroponics to observe the

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https://doi.org/10.1016/j.scienta.2024.113452
Received 12 March 2024; Received in revised form 13 June 2024; Accepted 28 June 2024
Available online 14 July 2024

root structure and the formation of adventitious roots (AR), which is the prerequisite for the vegetative propagation of herbaceous or woody horticultural crops (Sharma et al., 2018). Aeroponics has also been used to optimize clonal stem cutting propagation to ornamentals such as roses (Lee et al., 2006), petunia (Santos et al., 2009), Korean lilac and inkberry (Peterson et al., 2018), campanula (Bergonci and Paponov, 2022), and medicinal plants (Kumari et al., 2016). The advantages of aeroponics in cutting propagation have been presented by Mehandru et al. (2014) in studies on *Caralluma edulis, Leptadenia reticulata* and *Tylophora indica*. In all the species studied, the length and number of adventitious roots per cutting and the percentage of cuttings rooted aeroponically were significantly higher than the soil-grown stem cuttings. In a recent study by Weingarten et al. (2024), it was found that cuttings of *Cannabis sativa* propagated aeroponically performed as well as, and in some cases better than, those propagated in foam and rockwool soilless systems. This was particularly evident in the promotion of root growth, plant height, and both above-ground and root dry mass. Similarly, Martin-Laurent et al. (1997) found that Acacia mangium saplings grown aeroponically and inoculated with Bradyrhizobium spp. developed significantly more small nodules distributed across their root systems than those grown in liquid or sand media. This enhanced symbiosis increased nitrogen and chlorophyll content in the plant tissues.

Moreover, aeroponics could enable the cultivation of healthy and morphologically superior cuttings without using non-renewable or very slowly renewable resources such as peat (Giménez et al., 2019; Gruda, 2022; Gruda et al., 2019, 2024), in contrast to conventional methods. Additionally, it facilitates the easy identification and prevention of plant diseases (Buckseth et al., 2016). Closed recycled management delivers significant water savings compared to other cultivation systems (AlShrouf, 2017; Lakhiar et al., 2020). This technology exposes stem cutting to an aerosol solution during the spray time atomized directly through pressure, air-assist, or ultrasonic atomizers (Min et al., 2023) at predetermined spray intervals in which the solution is not delivered.

In aeroponics, choosing the proper duration of both spray and spray intervals, based on the plant requirements, is crucial for the success of the propagation (Lakhiar et al., 2018). Ikiz et al. (2020) reported that the highest biomass yield, including root, and the highest edible fresh onion yield were obtained from 18 s spray time with 10 min spray interval.

Another factor that affects clonal propagation is the pre-treatment with auxin which constitutes a group of low-weight molecules that induce growth and significantly affect plant development (Gomes and Scortecci, 2021). These plant hormones involve several critical biological processes, such as cell signaling, cell cycle regulation, embryogenesis, organogenesis, and growth regulation (Du et al., 2020). The most common auxin includes indoleacetic acid (IAA), indole-3-butyric acid (IBA), 4-chloroindole-3-acetic acid (4-Cl-IAA), and phenylacetic acid (PAA). IAA is the most abundant in plants (Gomes et al., 2021). In species with low internal auxin levels, exogenous auxin can be applied to prevent root death and improve both AR development and quality, thereby ensuring better success (Zheng et al., 2020). IBA and 1-naphthaleneacetic acid (NAA) are widely used to promote root development and quality during vegetative cutting propagation (Kyrkas et al., 2024). Auxin also enhances transplant success (Cano et al., 2018).

Considering our knowledge, there is still little information on ornamental sage's hydroponics multiplication. This research investigated the influence of the water spraying intervals and IBA concentrations for two commercial-relevant ornamental sage cultivars on AR development and quality of ground and above-ground rooted cuttings under an aeroponic clonal propagation system. We hypothesize a synergistic effect on root development between spray interval and IBA.

2. Materials and methods

2.1. Plant materials and rooting environment

The experiment was conducted during from March 21 st (cutting data) to April 20 th (cutting harvesting data) 2023, in a commercial nursery farm in Italy (40°54′19.1″ N, 17°18′21.4″ E) under an ethylene-vinyl acetate propagation greenhouse, equipped with thermal screens. The microclimatic parameters were monitored: air temperature ranged from 15±1.5 °C (minimum night temperature) to 25±1.5 °C (maximum day temperature), and air relative humidity was kept at 60±5% through an automated fog system.

The aeroponic propagation system comprised 12 propagation units 'X-Stream aero' (Nutriculture Ltd., UK), each measuring 115 × 64 × 46 cm and 70 L in volume. Each unit consisted of a reservoir on top of three plastic trays with 40 holes 3.5 cm in diameter for 120 holes (Fig. 1).

On March 21st, every hole was filled with a sponge disc to keep the cutting in place. A submersible pump with a timer on the bottom of the reservoir allowed the timed delivery of water spray and spray intervals through three 'H' shaped pressure atomizers.

The nursery farm provided the starting plant material for the experimental trial: 960 sub-apical, binodal sage cuttings, with an average length of 5 cm, presenting one pair of leaves each. The cuttings were excised from healthy, mature mother plants grown in the open air in a dedicated nursery area.

2.2. Experimental design

The treatments tested were: two different spraying intervals (I): 5 min (I_5) and 10 min (I_{10}), both with a 1 min spraying time (in which water is delivered); two IBA concentrations (C): C_0 (0 g L^{-1}) and C_1 (1 g L^{-1}); two cultivars: cv_1 'Farina Silver Blue' and cv_2 'La Siesta'.

Choudary and Kataria (2022) investigated the 10-minute spray interval as a single timing. In our research, we compared it with the spray interval of half the duration (I_5).

Both CVs are salvias of great commercial interest as they are used in Mediterranean public and private green areas because they are used in xeriscaping. Rooting of *S.* 'Farina Silver Blue' and 'La Siesta' cuttings has not yet been studied in aeroponics. Experimentation for each specific cultivar is necessary to determine the appropriate rooting IBA treatment since it is well established that exogenous application of auxin accelerates the rates of rooting, increases the final rooting percentage, and improves the number of AR in leafy cuttings (Blythe et al., 2004). Regarding IBA applied in higher concentrations than 1 gL^{-1} it may result in inhibiting rhizogenesis (McGuire et al., 1998), and IBA, at the dose of 1 gL^{-1} was effective in promoting AR formation in sage (*S. officinalis* L. and *S. triloba* L.) stem cuttings (Kaçar et al., 2009).

The basal end of the stem cuttings (around 1 cm) was dipped into the selected IBA (Sigma-Aldrich, St. Louis, MO, USA) concentrations (in solution) for 5 min and immediately positioned into the sponge discs in the plastic trays.

The experiment layout was a strip-split-plot design, with three replicates. Spraying intervals were represented in the main plots, and IBA

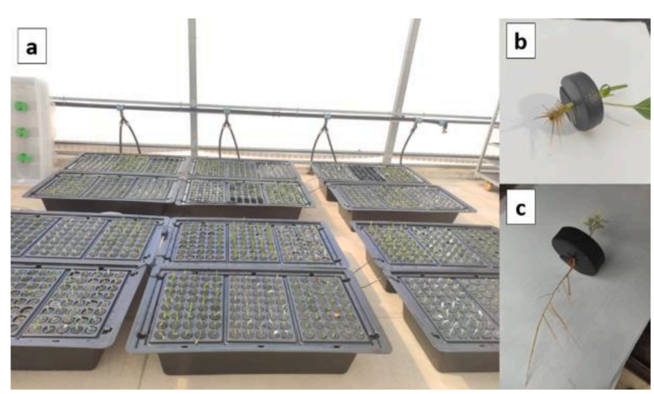

Fig. 1. | On the left front view (**a**) of the aeroponic propagation system, rooted cutting of cv1 'Farina Silver Blue' (**b**) and cv2 'La Siesta'(**c**).

concentrations and cultivars represented the sub-plots and sub-subplots, respectively. Each experimental unit contained 40 cuttings.

2.3. Adventitious root development and quality of ground and above-ground rooted cutting

The findings concerned AR development and quality traits of ground and above-ground rooted cuttings.

Cuttings (%) presenting first roots (any first roots that were >1 mm in length and easily visible with the naked eye) were taken at 7 DAC (Days After Cutting, March 28 th); rooted cuttings (%) and the number of roots/cutting (no) were taken at 21 DAC (April 11 th). On April 11 th the roots were scanned at 400 dpi using an Epson v700 Perfection (Japan) scanner. The captured images were then processed using image analysis software (WinRHIZO v. 2012b©, Regent Instruments Inc., Québec, QC, Canada, www.regentinstruments.com (lastly accessed on April 17, 2023) to determine root length (cm), surface area (cm2), average diameter (mm), volume (cm3), number of tips, forks and crossings.

Ground both fresh weight (g) and biomass (%) and dry weight (g) and dry matter (%) were recorded from April 17th to April 19th.

Roots dry weights were obtained by placing the collected fresh roots in the oven at 70±2 °C for 48 h.

Ground fresh biomass of the rooted cutting was calculated by the formula:

$$\frac{\textit{root fresh weight } (g)}{\textit{total fresh weight } (g)} * 100.$$

Ground dry matter was calculated by the formula:

$$\frac{\textit{root dry weight } (g)}{\textit{root fresh weight}(g)} * 100.$$

The above-ground biometric parameters of rooted cuttings: leaves/cutting (no), total fresh weight (g) and leaves fresh weight (g) were recorded too. Ground and above-ground fresh and dry weights were measured by an electronic analytical balance with an accuracy of 0.1 ± 0.1 mg.

2.4. Statistical analysis

A three-way ANOVA was performed to test the effects of spraying intervals (I), IBA concentration (C), cultivar (cv), and their interactions on recorded parameters. Before performing ANOVA, percentage data were arcsine transformed.

All data analyses were performed using SAS version 9.3 statistical software (Piepho, 1999); treatment means were separated by the L.S.D. (Least Significant Difference) test ($p \leq 0.05$). Means are presented with standard errors. Pearson's correlation analysis was carried out to evaluate the relationships between the morphological and productive parameters of sage-rooted cuttings.

3. Results

3.1. Adventitious root development

Table 1 reports the values of the main effects and interactions of the applied treatments on the percentage of cuttings with first roots, rooted cuttings, and the number of roots per cutting. Cuttings treated under spraying interval I_{10} exhibited higher values of cuttings with first roots (+33 %), rooted cuttings (+12 %), and roots number per cutting (+29 %), compared to those treated with I_5 spraying interval. IBA C_1 concentration (1 gL^{-1}) also gave significant results, with +81 % in cuttings with first roots, +36 % in rooted cuttings and +70 % in root number per cutting compared to the C_0 IBA-treated. Moreover, cv_1 'Farina Silver Blue' gave better overall results than 'La Siesta' (Table 1) regarding cuttings with first roots (+55 %) and 55 roots per cutting. Conversely, both cultivars gave similar results regarding the percentage of rooted cuttings.

Table 1
Main effects and interactions of spraying intervals (I), IBA concentration (C), and cultivar (*Salvia* 'Farina Silver Blue' (cv_1) and *Salvia* 'La Siesta' (cv_2) on rooted cuttings development.

Treatments	Cuttings with first roots (%) (1)	Rooted cuttings (%) (2)	Roots/cutting (no)(2)
Spraying intervals (I)			
I_5	19.5 ± 6.7b	68.1 ± 4.5b	8.2 ± 1.5b
I_{10}	29.3 ± 6.9a	77.3 ± 4.2a	11.6 ± 2.7a
IBA concentration (g L^{-1}) (C)			
C_0	7.9 ± 2.1b	55.8 ± 2.1b	4.7 ± 0.4b
C_1	40.8 ± 6.6a	86.6 ± 1.6a	15.1 ± 2.2a
Cultivar (cv)			
cv_1	33.6 ± 8.2a	71.8 ± 4.4a	13.6 ± 2.6a
cv_2	15.1 ± 3.8b	73.6 ± 4.7a	6.2 ± 0.9b
Significance			
I	*	**	**
C	**	**	**
cv	**	Ns	**
I x C	**	**	**
I x cv	Ns	Ns	*
C x cv	**	**	**
I x C x cv	Ns	Ns	ns

Different letters within each column indicate significant differences according to the LSD test ($P \leq 0.05$; mean ± SD, $n = 12$). The statistical significance is designated by asterisks as follows: *, at $p \leq 0.05$; **; at $p \leq 0.01$; ns = not significant. (1) Mean recorded value at 7DAC, (2) Mean values recorded at 21 DAC.

Significant interaction effects were found for spraying intervals*IBA concentration (Fig. 2, a-c): best percentage of cuttings with first roots (a) were obtained for both spraying intervals under C_1 IBA (44 and 38 %, respectively). At I_{10} *C_1, 91 % of the cuttings were successfully rooted, with an average of 18 roots per cutting (Fig. 2, b-c). Moreover, IBA*cv interaction was significant for the traits reported previously in Table 1.

Fig. 3 (a-c) shows that higher values of cuttings with first roots (a) and roots per cutting (c) were obtained from cv_1 under C_1 IBA (59 % and 21 roots); regarding the percentage of rooted cuttings (b), higher values were achieved for both cultivar under C_1 IBA treatments (85 and 88 %, respectively in cv_1 and cv_2).

3.2. Quality of ground-rooted cutting

Table 2 reports the main effects and interactions of the spraying intervals, IBA concentrations, and cultivars on cuttings' root morphological characteristics.

The root length significantly increased in cuttings treated with I_{10} spraying interval (+52 %) compared to I_5-treated cuttings; the same trend was observed in surface area (+59 %), volume (+50 %), and number of tips (+59 %), forks (+58 %) and crossings (+73 %). Cuttings under C_1 showed only increased root length values (+15 %) when compared to C_0 IBA; better results regarding root diameter (+17 %), volume (+29 %), and tips number (+18 %) were achieved by cuttings treated with C_0 IBA.

The cultivar factor also significantly affected several characteristics: cv_2 'La Siesta' scored better results overall compared to cv_1 'Farina Silver Blue' regarding root length (+34 %), surface area (+22 %), volume (+50 %), number of tips (+50 %), forks (+55 %) and crossings (+31 %) when compared to cv_1 (Table 2).

Furthermore, data analysis showed a significant positive interaction between spraying intervals and IBA concentration for all the mentioned previously traits (Table 2, Fig. 4). Fig. 4 (a-d) shows that cuttings under I_{10} spraying interval gave significantly better results for (a) root length compared to I_5 regardless of IBA concentration (55 and 59 cm respectively in C_0 and C_1); this trend was repeated for the (b) surface area (C_0: 10.3 and C_1: 10.9cm^2). Higher root diameter (c) and volume (d) values

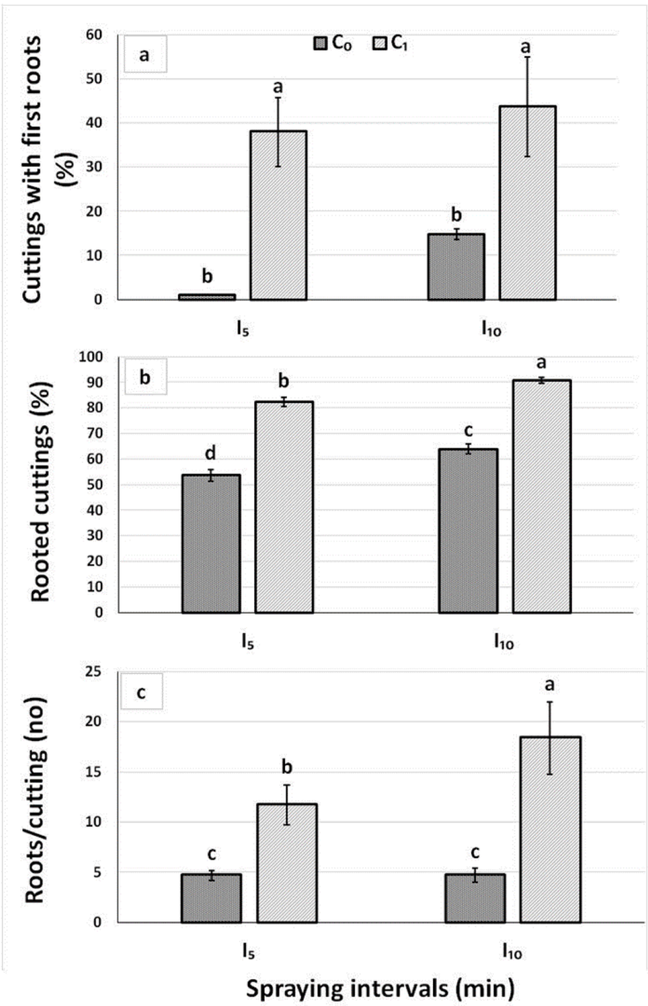

Fig. 2. | Effect of spraying intervals (I_5 and I_{10}) and IBA concentration (C_0, and C_1) on cuttings with first roots 7DAC (**a**), rooted cuttings 21DAC (**b**) and roots/cutting (no) 21DAC (**c**). Vertical bars (standard error) (n = 12) with different letters are significantly different according to the LSD test (P = 0.05).

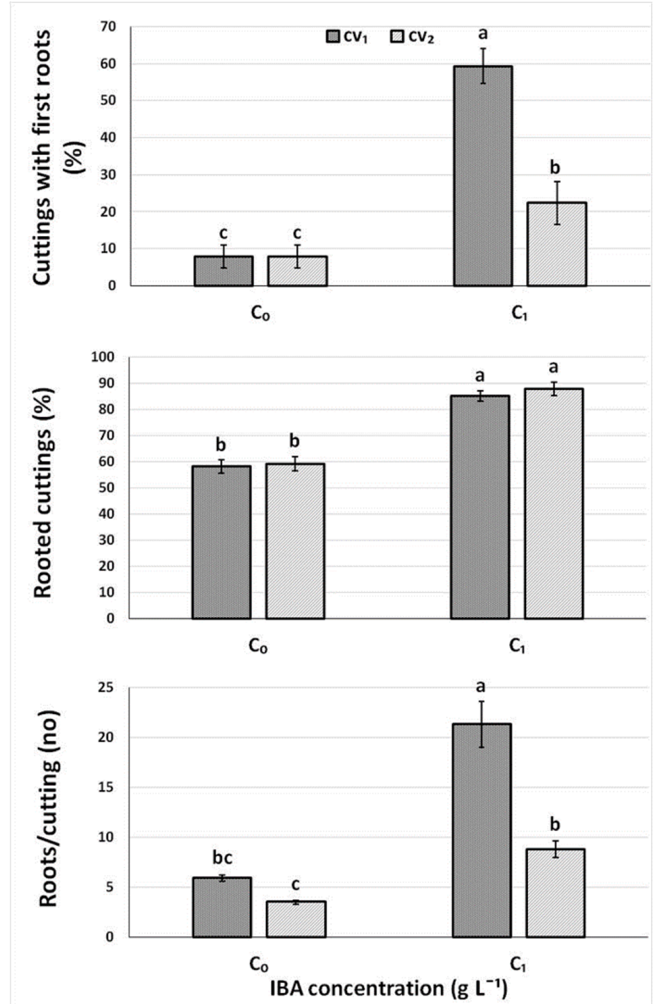

Fig. 3. | Effect of IBA concentration (C_0, and C_1) and cultivar (cv_1= Salvia 'Farina Silver Blue'; cv_2 = Salvia 'La Siesta') on cuttings with first roots 7DAC (**a**), rooted cuttings 21DAC (**b**) and roots/cutting (no) 21DAC (**c**). Vertical bars (standard error) (n = 12) with different letters are significantly different according to the LSD test (P = 0.05).

were instead recorded for cuttings treated with C_0 IBA under I_{10} (0.6 mm and 0.17 cm^3).

Fig. 5 (a-c) represents the interactions for the number of tips (a), forks (b) and crossings (c). In terms of the number of tips, cuttings treated with C_0 IBA under I_{10} performed better (75.5); while, for forks and crossings, the best results were obtained under I_{10} regardless of the IBA concentration (44.9 and 48 for forks and 33.1 and 35.5 for crossings).

The effect of the interaction between spraying interval and cultivars was also significant for almost all the root morphological traits (Tables 2 and 3). Table 3 shows that better values were achieved overall by cv_2 under I_{10} spraying interval for length (66.2 cm), volume (0.2cm^3), number of tips (86.0), forks (63.6) and crossings (44.4). Regarding surface area, higher results were achieved in cuttings under I_{10} for both cultivars (10.2 and 11.1 cm^2). Finally, spraying interval*cultivar interaction was found to be significant for the roots/cutting parameter (Table 3) with better values recorded for cv_1 (16.3 and 11.0 %).

The interaction IBA concentration*cultivar was significant only for root diameter (a) and volume (b): in both cases better results were achieved by C_0 IBA in cv_2 cuttings (0.6 mm and 0.17cm^3, respectively) (Fig. 6, a-b).

Table 4 displays the ground fresh and dry weights, fresh biomass, and dry matter percentages. Cuttings treated with I_{10} showed a 23 % increase in root fresh weight, dry weight, and biomass percentage compared to cuttings treated with I_5. Similarly, cuttings treated with C_1 IBA showed a 50 % increase in fresh weight, a 55.5 % increase in dry weight, and a 36.8 % increase in biomass percentage compared to C_0-treated cuttings. Among the two cultivars, cv_1 performed better in terms of root fresh weight (32 %) and dry weight (14 %), while cv_2 gave better results in root dry matter (21 %) and biomass (43 %).

The effect of the interaction between spraying intervals and IBA concentrations was significant for root fresh biomass% (a) and dry weight (b) with higher values for C_1 IBA-treated cuttings under I_{10}: 37 % and 0.29 g, respectively (Fig. 7, a-b).

Interaction between irrigation timings and cultivar was found to be significant for root biomass and dry matter (Fig. 8, a-b): more specifically, cv_2 developed higher values for root biomass (35 and 36 %) and root dry matter (6.9 and 8.0 %) regardless the spraying interval.

The interaction IBA concentrations*cultivars gave significant values for all the parameters in the exam (Fig. 9a-d). Cuttings treated with C_1 IBA concentration gave generally better results compared to the C_0-treated ones. More specifically, IBA-treated cv_1 cuttings gave better results (4.7 g) for root fresh weight (a) values, while statistically similar results were obtained in both cultivars for root dry weight (b) (0.28 g and 0.25 g, respectively cv_1 and cv_2). On the other side, C_1 IBA-treated cv_2 cuttings showed higher values for root biomass (c) and dry matter (d) (42.3 and 8.3 %) than C_1 IBA-treated cv_1 cuttings.

Table 2
Main effects and interactions of spraying intervals (I), IBA concentrations (C), and cultivar (*Salvia* 'Farina Silver Blue' (cv_1) and *Salvia* 'La Siesta' (cv_2) on root morphological traits at 21 DAC.

Treatments	Root						
	Length (cm)	Surface area (cm^2)	Diameter (mm)	Volume (cm^3)	Tip numbers	Fork numbers	Crossings numbers
Spraying intervals (I)							
I_5	27.6 ± 3.0b	4.4 ± 0.5b	0.50±0.01a	0.07±0.01b	26.4 ± 3.1b	19.4 ± 2.8b	9.4 ± 2.0b
I_{10}	57.0 ± 3.7a	10.6 ± 0.1a	0.52±0.03a	0.13±0.02a	65.1 ± 9.0a	46.4 ± 7.7a	34.3 ± 3.7a
IBA concentration (g L^{-1}) (C)							
C_0	38.8 ± 6.1b	7.3 ± 1.3a	0.60±0.02a	0.12±0.02a	50.1 ± 11.6a	30.2 ± 9.0a	20.1 ± 5.2b
C_1	45.9 ± 3.7a	7.7 ± 1.2a	0.50±0.01b	0.08±0.01b	41.3 ± 4.5b	35.6 ± 4.3a	23.6 ± 4.2a
Cultivar (cv)							
cv_1	33.7 ± 4.6b	6.6 ± 1.3b	0.52±0.02a	0.07±0.01b	30.4 ± 4.6b	20.4 ± 4.3b	13.9 ± 3.5b
cv_2	51.0 ± 5.1a	8.5 ± 1.1a	0.50±0.03a	0.13±0.02a	61.0 ± 9.7a	45.4 ± 7.3a	29.8 ± 4.6a
Significance							
I	**	**	ns	**	**	**	**
C	*	Ns	***	**	**	Ns	*
Cv	**	*	ns	**	**	**	**
I x C	**	**	**	**	**	*	**
I x cv	**	**	ns	**	**	**	**
C x cv	ns	Ns	**	**	ns	ns	ns
I x C x cv	ns	ns	ns	ns	ns	ns	ns

Different letters within each column indicate significant differences according to the LSD test ($P \leq 0.05$; mean ± SD, $n = 12$). The statistical significance is designated by asterisks as follows: *, at $p \leq 0.05$; **; at $p \leq 0.01$; ns = not significant.

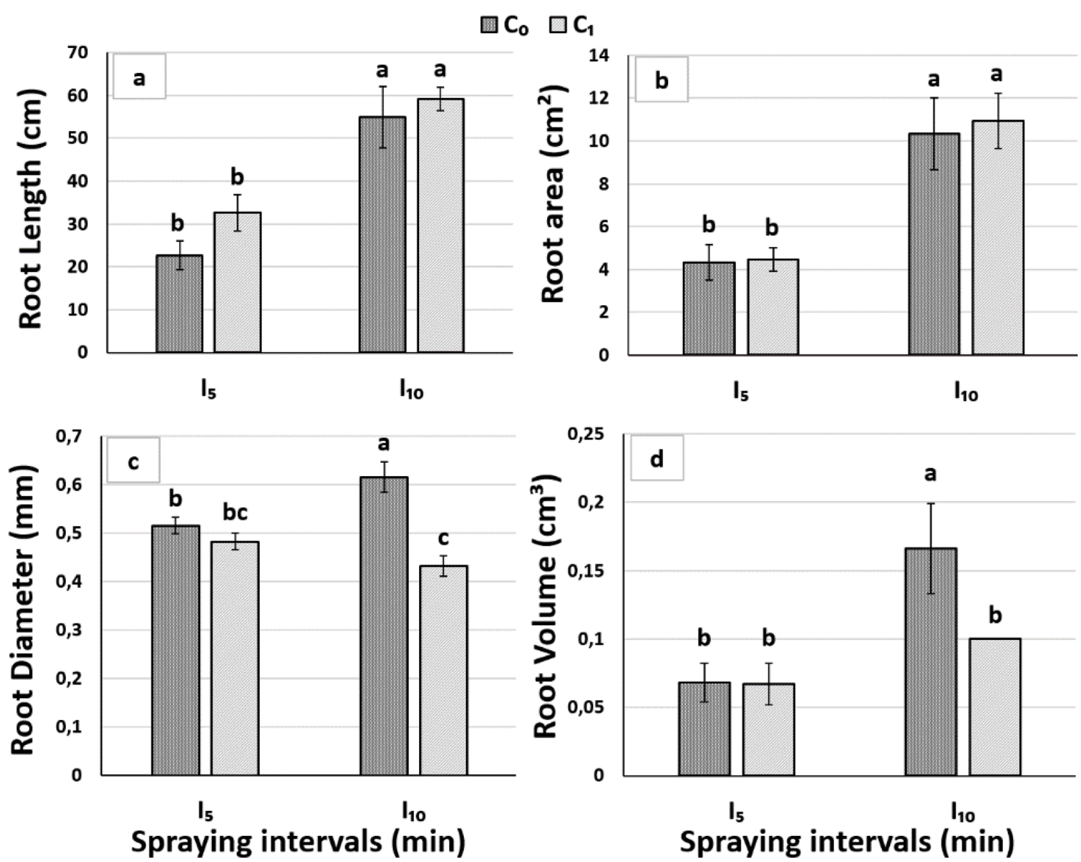

Fig. 4. | Effect of IBA concentration (C_0, and C_1) and spraying intervals (I_5 and I_{10}) on root length (cm) (**a**), surface area cm^2) (**b**), diameter (mm) (**c**) and volume (cm^3) (**d**) at 21DAC. Vertical bars (standard error) ($n = 12$) with different letters are significantly different according to the LSD test ($P = 0.05$).

3.3. Quality of above-ground rooted cutting

Table 5 reports the main effects and interactions of the treatments applied for the above-ground cuttings parameters. I_{10}-treated cuttings showed homogeneously better results than I_5-treated cuttings for all the examined parameters (+9 % as leaves number, +11 % as total fresh weight, and +5 % as leaves fresh weight). C_1 IBA-treated cuttings gave better results for the total fresh weight (+12 %) and leaves fresh weight (+9 %) compared to the C_0-treated cuttings, which exhibit a higher value for leaves number (+9 %) compared to the C_1 IBA-treated cuttings. Cv_1 performed better than cv_2 with higher values for total fresh (+71 %) and leaves weights (+66 %).

The interaction spraying intervals*cultivars (Fig. 10) was significant for fresh total and leaves weight: cv_1 cuttings treated with I_{10} spraying

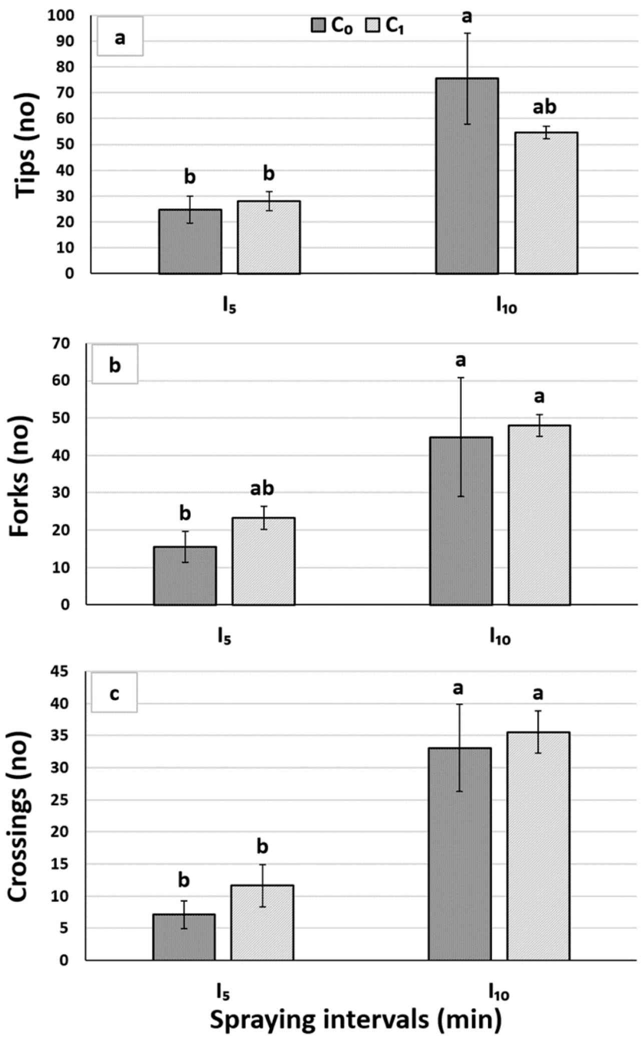

Fig. 5. | Effect of spraying intervals (I_5 and I_{10}) and IBA concentration (C_0, and C_1) on the number of tips (**a**), forks (**b**) and crossings (**c**) at 21DAC. Vertical bars (standard error) (n = 12) with different letters are significantly different according to the LSD test ($P = 0.05$).

interval scored higher values for the total fresh weight (14.2 g) and leaves fresh weight (6.3 g).

The same significant interactions were obtained from IBA concentration*cultivars for total and leaf fresh weight (Fig. 11). For leaves fresh weight, cv_1 cuttings showed better results when treated with C0 IBA (8.3 g). For total fresh weight, higher values were obtained by cv_1 cuttings regardless of the irrigation timing (16.3 g and 18.0 g), with slightly higher values for I_{10}-treated cuttings.

3.4. Pearson correlation analysis

Correlation analysis (Table 6) on morphological and productive parameters of rooted sage cuttings showed that root number per cutting was significantly correlated with ground fresh weight (r = 0.911), ground dry weight (r = 0.685), and total fresh weight (r = 0.776). Root surface area was positively correlated with root length (r = 0.875) and a positive correlation was found between ground dry and fresh weight (r = 0.830).

Finally, no negatively significant correlation was found between the parameters in the exam.

4. Discussion

Adventitious root formation plays a pivotal role in propagating plants through cuttings, contributing significantly to their overall growth and establishment. This natural process involves the development of roots from non-root tissues, such as stems or leaves, and is crucial for the successful cloning and reproduction of many plant species. (Steffens and Rasmussen, 2016; Druege et al., 2019). Aeroponic propagation has been successfully used on numerous vegetable crops such as lettuce (Li et al., 2018), tomato (Wang et al., 2019), potato (Tunio et al., 2020), *Brassica* spp. (Srikanth et al., 2016), strawberry (Kanechi et al., 2015) and cannabis (Ferrini et al., 2021).The correlation between different spraying intervals and vegetable crop growth in aeroponics has been addressed in works on onion (Ikiz et al., 2018), potatoes (Tengli and Raju, 2022), and lettuce (Tunio et al., 2021).

Concerning ornamentals, researches addressing cutting in aeroponics include chrysanthemums (Fanourakis et al., 2009), and roses (Jowkar et al., 2010). The correlation between IBA application and cutting rooted in aeroponics has been explored in numerous works on officinal plants (Mehandru et al., 2014; Sharma et al., 2018; Yachyaa et al., 2020; Choudhary and Kataria, 2022), vegetable crops (Regas et al., 2021; Nishchitha et al., 2023) and ornamentals (Peterson et al., 2018; Traykova and Stanilova, 2020). Based on the literature, very few researchers conducted studies on the effects of spraying interval and IBA concentration on AR development and quality in aeroponic systems applied to cutting propagation.

Aeroponics is the most popular hydroponics system in the world (Rajendran et al., 2024). Our findings showed that cuttings treated under spraying interval I_{10} exhibited higher percentages of cuttings with first roots, rooted cuttings, and roots per cutting compared to those treated with I_5 spraying interval (Table 1). In the absence of water spray, the oxygen has a contact more continuous with cutting, and stimulates metabolic processes, positively affecting the development of AR and shoots. Furthermore, a study by Tunio et al. (2022) reported that constant atomization contributed to fungal and bacterial growth in plants. In our research, the I_{10} spray interval improved all morphological parameters of the root, compared to I_5 (Table 2). Root formation in chrysanthemum cuttings with root spraying has been reported by Molitor and Fischer (1993), who used relatively short spray time (30 s) and relatively long spray intervals (up to 4 h). I_{10} improved both fresh and

Table 3
Effects of the interactions of spraying intervals (I_5 and I_{10}) and cultivar (*Salvia* 'Farina Silver Blue' (cv_1) and *Salvia* 'La Siesta' (cv_2) on root morphological traits at 21DAC.

Treatments		Root						
		Roots /cutting (no)	Length (cm)	Surface Area (cm^2)	Volume (cm^3)	Tips (no)	Forks (no)	Crossings (no)
I_5	cv_1	11.0 ± 2.4a	19.6 ± 2.0d	2.9 ± 0.2b	0.03±0.01c	16.7 ± 1.7c	11.5 ± 2.2b	3.6 ± 0.4d
	cv_2	5.5 ± 0.9b	35.7 ± 3.0c	5.8 ± 0.3b	0.10±0.01b	36.0 ± 1.7bc	27.3 ± 1.9b	15.2 ± 1.9c
I_{10}	cv_1	16.3 ± 4.5a	47.8 ± 3.9b	10.2 ± 1.5a	0.10±0.01b	44.1 ± 4.1b	29.3 ± 6.8b	24.2 ± 3.4b
	cv_2	6.8 ± 1.6b	66.2 ± 3.9a	11.1 ± 1.5a	0.17±0.03a	80.0 ± 12.9a	63.6 ± 9.8a	44.4 ± 2.5a

Different letters within each column indicate significant differences according to the LSD test ($P \leq 0.05$; mean ± SD, n = 12). The statistical significance is designated by asterisks as follows: *, at $p \leq 0.05$; **; at $p \leq 0.01$; ns = not significant.

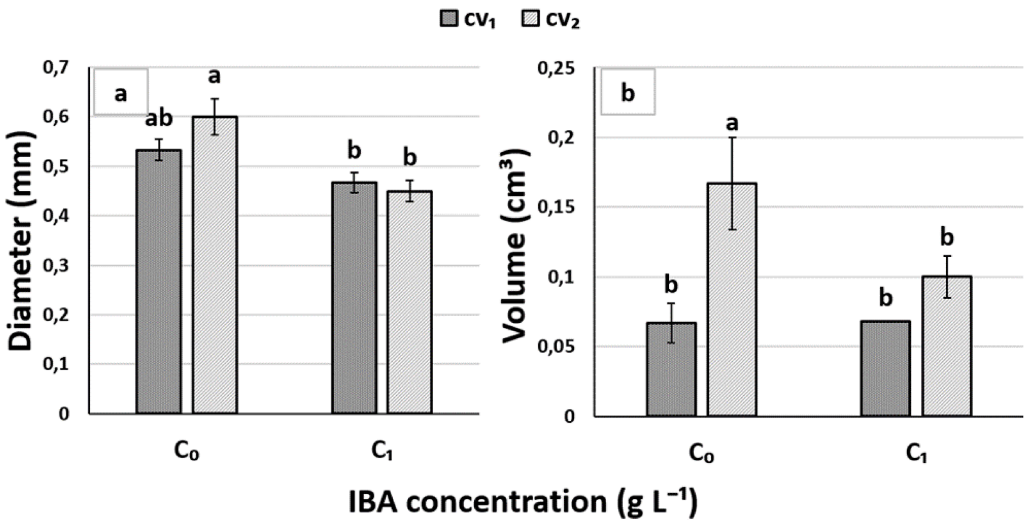

Fig. 6. | Effect of IBA concentration (C_0, and C_1) and cultivars (cv_1= *Salvia* 'Farina Silver Blue'; cv_2= *Salvia* 'La Siesta') on root diameter (mm) (**a**) root volume (cm^3) (**b**) at 21DAC. Vertical bars (standard error) (n = 12) with different letters are significantly different according to the LSD test (P = 0.05).

Table 4
Main effects of spraying intervals (I), IBA concentration (C) and cultivar (*Salvia* 'Farina Silver Blue' (cv_1) and *Salvia* 'La Siesta' (cv_2) on ground fresh and dry weights, fresh biomass and dry matter parameters at 21DAC.

Treatments	Ground			
	Fresh		Dry	
	Weight (g)	Biomass (%)	Weight (g)	Matter (%)
Spraying intervals (I)				
I_5	2.3 ± 0.3b	25.9 ± 3.2b	0.17±0.02b	6.8 ± 0.4a
I_{10}	3.4 ± 0.5a	29.9 ± 3.2a	0.22±0.02a	6.6 ± 0.4a
IBA concentration (g L^{-1}) (C)				
C_0	1.9 ± 0.2b	21.6 ± 2.6b	0.12±0.01b	6.4 ± 0.5a
C_1	3.8 ± 0.4a	34.2 ± 2.7a	0.27±0.01a	7.0 ± 0.5a
Cultivar (cv)				
Cv_1	3.4 ± 0.5a	20.2 ± 2.3b	0.21±0.02a	5.9 ± 0.4b
Cv_2	2.3 ± 0.2b	35.6 ± 2.3a	0.18±0.02b	7.5 ± 0.4a
Significance				
I	**	**	**	ns
C	**	**	**	ns
cv	**	**	*	**
I x C	**	*	**	ns
I x cv	*	**	ns	**
C x cv	**	**	**	*
I x C x cv	Ns	ns	ns	ns

Different letters within each column indicate significant differences according to the LSD test ($P \leq 0.05$; mean ± SD, n = 12). The statistical significance is designated by asterisks as follows: *, at $p \leq 0.05$; **; at $p \leq 0.01$; ns = not significant.

dry root weight.

Regarding nutrient delivery interval in roses growing in aeroponics, a 15-minute spray interval improved the length and thickness of the flowering stem and the total leaf number (Jowkar et al., 2010), according to what is shown in Table 5.

Adventitious root AR initiation can be stimulated by auxin (Omary et al., 2023); but at high concentrations, it generally disturbs root elongation and instead increases AR formation (Kim et al., 2021). In our research, IBA applied at 1 g L^{-1}, had a positive impact on AR development (Table 1). Nanos et al. (2023) showed that K-IBA 0.5 and 1.0 g L^{-1} concentrations were similarly effective in sage rooting percentage. Furthermore, the same authors highlighted that the number of roots increased significantly in the presence of K-IBA compared to the control, especially at 1 gL^{-1}, in agreement with our results. On the contrary, Bertsouklis et al. (2022) found that the highest rooting percentage of terminal cuttings in Salvia fruticosa was 55 % and was achieved after treatment with 4500 mg L^{-1} IBA. In our research, IBA did not have a remarkable effect on root morphology traits such as surface area and crossings or even worsened cutting performance of diameter, volume, and tips (Table 2). In our study, the lack of the IBA effect or worsening because of its application may indicate that a greater concentration (> 1 g L^{-1}), an alternative mode (powder), and duration of application (more than 5 min) may be necessary for sage rooting. The dry mass of roots produced per rooted cutting was significantly increased with increasing concentration of IBA compared to control (Table 4). Bertsouklis et al. (2022) found that basal cuttings harvested from mother plants grown in the greenhouse showed no differences in the fresh and dry weights of roots.

By comparison between the two sage CV's, the rooting% was similar; on the other hand, cv_1 produced twice as many roots as cv_2 (Table 1). The latter was characterized by a better root morphology in terms of length, area, tips, forks, and crossings (Table 2). The ground fresh biomass (%) and dry matter content (%) of cv_2 also increased compared to cv_1 (Table 4).

Regarding the interaction effects in this research, it does not appear possible to make a comparison with other research that took into consideration only one of the two variabilities analyzed (IBA concentrations or spraying intervals). However, regarding chrysanthemum, Fanourakis et al. (2009) tested three different watering intervals (12 × 1, 3 × 2, and 1 × 6 times × min/h) on qualitative parameters of cuttings grown in aeroponics. Significantly higher total shoot (0.38 g) and root (0.065 g) dry weights per plant were recorded in cuttings misted with 3 × 2′ frequency that induced optimum plant growth with half the total amount of water used in 12 × 1′. Although numerous experiments have used IBA in aeroponic cultivation for propagating cuttings of vegetable crops (Del Valle-Echevarria et al. 2019; Wasilewska-Nascimento et al., 2020), few of these have investigated the AR development caused by the use of exogenous phytoregulators in ornamentals (Lee et al., 2006; Santos et al., 2009; Peterson et al., 2018). Mehandru et al. (2014); Sharma et al. (2018); Yachyaa et al. (2020), and Choudhary and Kataria (2022) investigated the effects of various concentrations of IBA alone or mixed with other compounds in various concentrations on the growth of medicinal plants cuttings in aeroponics. The results of these studies are not uniform and demonstrate a species-specific rooting response to the application of exogenous phytoregulators. For example, Mehandru et al. (2014), reports that *Caralluma edulis* showed higher root development (rooting%, roots/cuttings, and root length at IBA concentrations of 2 g L^{-1} (100 %, 8 and 3.5 cm), while *Leptadenia reticulata* and *Tylophora*

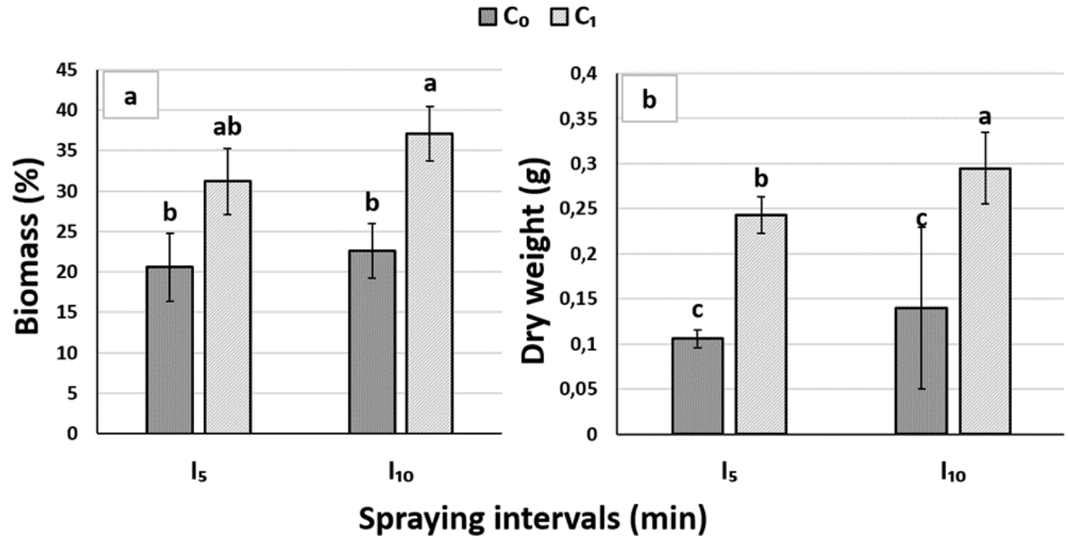

Fig. 7. | Effect of spraying intervals (I_5 and I_{10}) and IBA concentration (C_0, and C_1) on root fresh biomass (%) (**a**) and root dry weight (g) (**b**) at 21DAC. Vertical bars (standard error) (n = 12) with different letters are significantly different according to LSD test (P = 0.05).

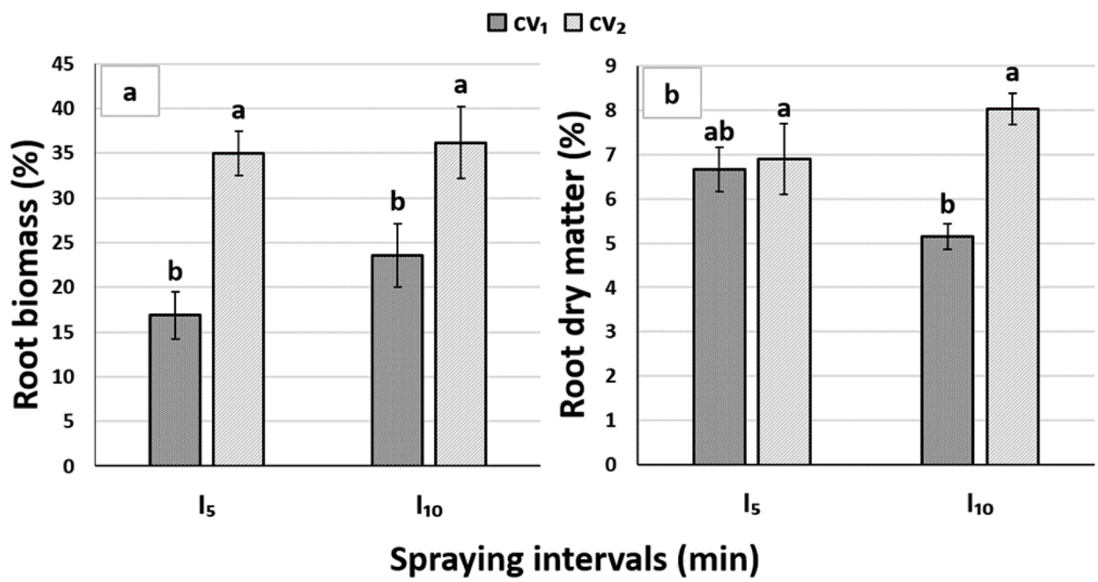

Fig. 8. | Effect of spraying intervals (I_5 and I_{10}) and cultivars (cv_1= *Salvia* 'Farina Silver Blue'; cv_2= *Salvia* 'La Siesta') on root fresh biomass (%) (**a**) and root dry matter (%) (**b**) at 21DAC. Vertical bars (standard error) (n = 12) with different letters are significantly different according to the LSD test (P = 0.05).

indica had optimal parameters at concentrations of 3 g L^{-1} IBA (97.7 %, 46.6 and 5.1 cm and 93.3 %, 23.7 and 0.89 cm, respectively).

Although Sharma et al. (2018) in *Tamarix aphylla*, found root development (rooting%, roots/cutting and root length) generally higher in cuttings treated with 2 g L^{-1} IBA (79 %, 9.4, and 8.2 cm), they also noted that the highest rooting values (87 %) was performed in cuttings treated with a mixture of IBA and NAA (2 g + 1 g L^{-1}). This variability in response to different phytohormones is also found in the work of Choudary and Kataria (2022) on *Sarcostemma acidum*, in which cuttings treated with different concentrations of IBA and NAA provide comparable, optimal adventitious rooting values at 3 g L^{-1} concentrations of phytoregulators. Specifically, the values were slightly higher for AR formation (90 %) and AR number/cutting (14) for cuttings treated with NAA (3 g L^{-1}) compared to 3 g IBA-treated cuttings (80 % and 12) while slightly higher values for root length (8.8 cm) were found in cuttings treated with IBA (3 g L^{-1}) compared to 3 g NAA-treated cuttings (7.1 cm). However, in contrast to these results, Yachyaa et al. (2020) observed that cuttings treated with 0 mg l-1 IBA+0.01 mg l-1 ethephon showed shorter times (9 days) for root emergence in their study on *Talinum paniculatum*, compared to those treated with IBA alone and the control.

Furthermore, cuttings treated with IBA alone at various doses (1, 2, 4 mg L^{-1}) showed a reduction of root length and biomass values. However, the application of IBA had a positive effect on root number with higher values (85) found in cuttings treated with 4 mg L^{-1} IBA+ 0 mg ethephon.

In conclusion, while there are some conflicting results, the majority of the cited research indicates a general positive effect of IBA applications on the root development of cuttings grown in aeroponics that aligns with our results. These results can be related to the positive outcomes found from the application of longer spray intervals on root development and allow us to hypothesize a synergistic effect on root development due to the combination of the two factors, spray interval and IBA.

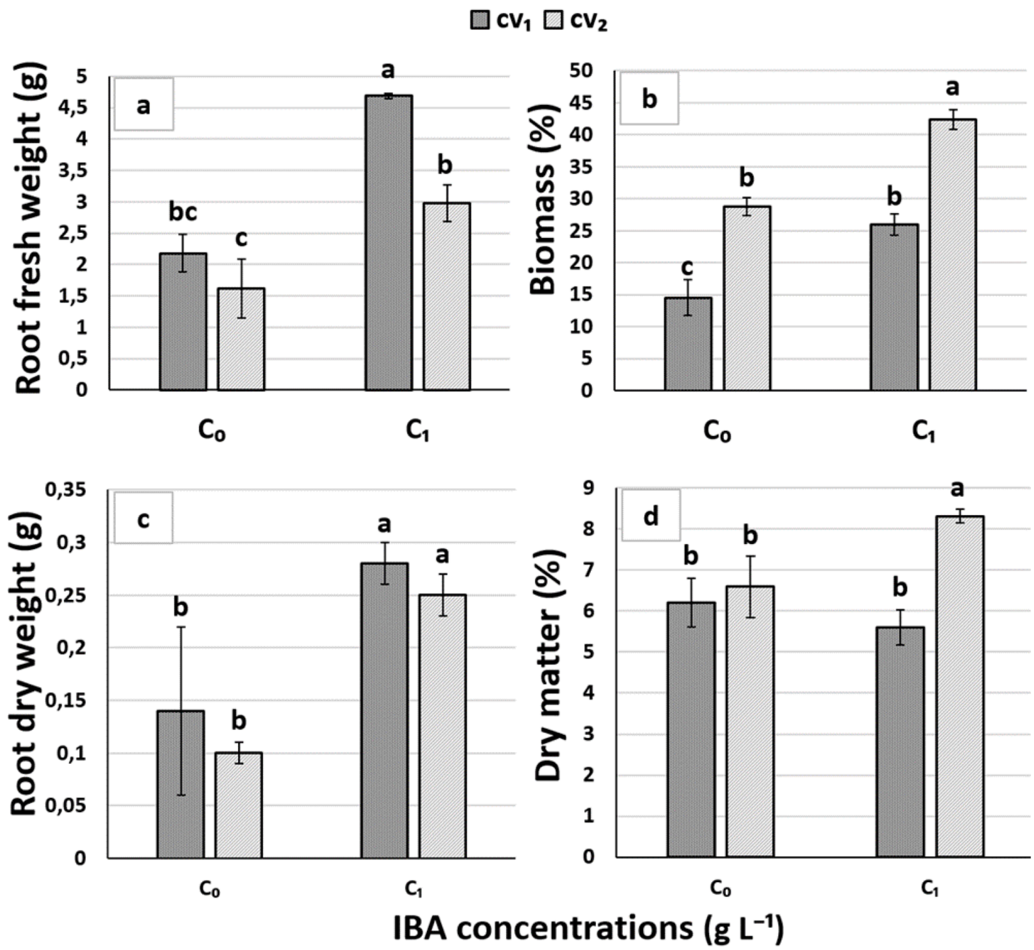

Fig. 9. | Effect of IBA concentration (C_0, and C_1) and cultivars (cv_1= *Salvia* 'Farina Silver Blue'; cv_2= *Salvia* 'La Siesta') on root fresh biomass (**a**), root fresh weight (g) (**b**), root dry matter (%) (**c**) and root dry weight (**d**) at 21DAC. Vertical bars (standard error) (n = 12) with different letters are significantly different according to the LSD test (P = 0.05).

Table 5
Main effects of Spraying intervals (I), IBA concentration (C) and cultivar (*Salvia* 'Farina Silver Blue' (cv_1) and Salvia 'La Siesta' (cv_2) on above-ground biometric parameters of cuttings.

Treatments	Leaves/cutting (no)	Total fresh weight (g)	Leaves fresh weight (g)
Spraying intervals (I)			
I_5	9.3 ± 0.3b	8.2 ± 1.5b	4.0 ± 0.6b
I_{10}	10.2 ± 0.3a	9.5 ± 1.8a	4.2 ± 0.6a
IBA concentration (g L^{-1}) (C)			
C_0	10.2 ± 0.3a	8.3b±*b*	3.9 ± 0.6b
C_1	9.2 ± 0.4b	9.4a±*a*	4.3 ± 0.6a
Cultivar (cv)			
Cv_1	10.0 ± 0.3a	13.7 ± 0.6a	6.1 ± 0.1a
Cv_2	9.5 ± 0.4a	4.0 ± 0.3b	2.1 ± 0.1b
Significance			
I	*	**	*
C	*	**	**
Cv	Ns	**	**
I x C	Ns	ns	ns
I x cv	Ns	**	**
C x cv	Ns	**	**
I x C x cv	Ns	ns	ns

Different letters within each column indicate significant differences according to the LSD test ($P \leq 0.05$; mean ± SD, n = 12). The statistical significance is designated by asterisks as follows: *, at $p \leq 0.05$; **; at $p \leq 0.01$; ns = not significant.

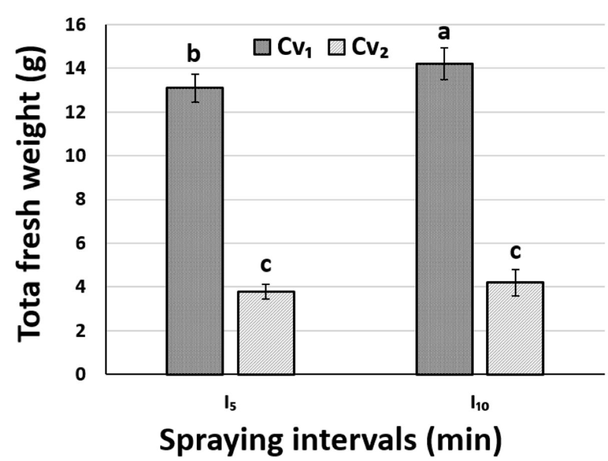

Fig. 10. | Effect of spraying intervals (I_5 and I_{10}) and cultivars (cv_1= *Salvia* 'Farina Silver Blue'; cv_2= *Salvia* 'La Siesta') on above-ground weight (g) at 21DAC. Vertical bars (standard error) (n = 12) with different letters are significantly different according to the LSD test (P = 0.05).

5. Conclusions

Sage cuttings grown in aeroponic performed better when pre-treated with exogenous auxin (IBA). Generally, cuttings grown with a spray

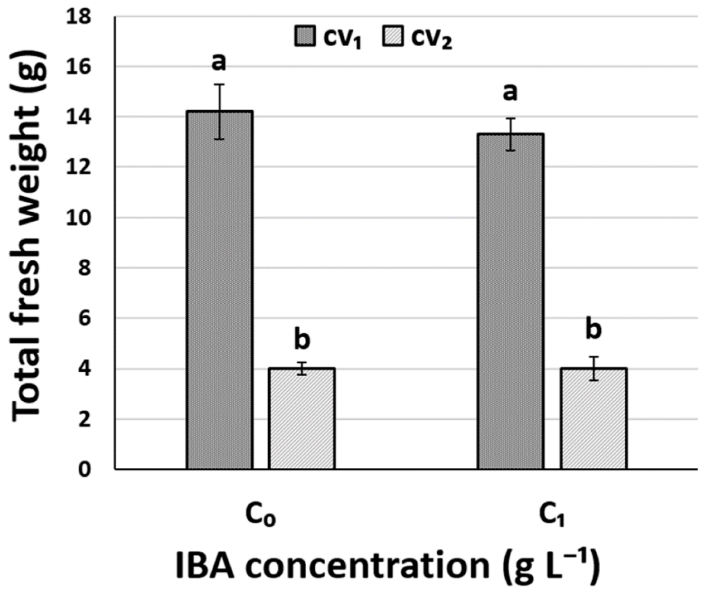

Fig. 11. |Effect of IBA concentration (C_0, and C_1) and cultivars (cv_1= *Salvia* 'Farina Silver Blue'; cv_2= *Salvia* 'La Siesta') on above-ground weight (g) at 21DAC. Vertical bars (standard error) (n = 12) with different letters are significantly different according to the LSD test (P = 0.05).

Table 6
Pearson correlation coefficients in morphological and productive parameters of rooted sage cuttings.

	Root cutting (no)	Root length (cm)	Root surface area (cm^2)	Leaves per cutting	Ground fresh weight (g)	Ground dry weight (g)	Total fresh weight (g)
Root cutting (no)							
Root length (cm)	-0.025						
Root surface area (cm^2)	0.164	0.875**					
Leaves per cutting	0.179	0.180	0.333				
Ground fresh weight (g)	0.911**	0.258	0.337	0.157			
Ground dry weight (g)	0.685**	0.274	0.145	-0.177	0.830**		
Total fresh weight (g)	0.776**	0.296	-0.093	0.252	0.615	0.356	

** significant at 0.01 probability level (n = 24).

interval I_{10} have shown higher quality parameters than those grown in I_5. According to statistical analysis, these results were found to correlate with IBA pre-treatment, allowing us to hypothesize a synergistic action between the spray interval I_{10} and the exogenous auxin application that, by taking advantage of aeroponic propagation, enables us to obtain healthy and morphological high-quality cuttings, without consumption of unremovable resources, i.e., peat, compared to the conventional method. In addition, no fertilizer it was used. These findings indicate the potential for sustainable cutting production. Furthermore, aeroponics may be a foundational technology for emerging systems, such as vertical farming.

CRediT authorship contribution statement

Eugenio Scaltrito: Writing – original draft, Writing – review & editing. **Giuseppe Cristiano:** Conceptualization, Writing – review & editing. **Anna Elisa Sdao:** Writing – review & editing. **Nazim S. Gruda:** Writing – review & editing. **Danilo Loconsole:** Writing – review & editing. **Barbara De Lucia:** Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Eugenio Scaltrito reports financial support was provided by Apulia Region Department of Agriculture Rural and Environmental Development. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This research was carried out with the contribution of POC Puglia FESR-FSE 2014 / 2020 Fondo Sociale Europeo approvato con Decisione C (2015)5854 del 13/08/2015 RIPARTI (assegni di RIcerca per riPARTire con le Imprese) Titolo del Progetto: "Produzione sostenibile e di qualità di materiale vivaistico ornamentale in aeroponica e in contenitore con l'uso di biostimolanti – Viv.aero.bios". Codice Pratica: 22/11481- Co - Bando D.R. n. 2643 del 19/07/2022 - programma n. 07.225 (In italian).

The authors thank Vivai Capitanio (Monopoli, BA, Italy) and agronomist Giovanni Fanizzi for providing the propagation material used in this research.

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