horticulturae

MDPI

Article

Asexual Propagation of Greek *Salvia officinalis* L. Populations Selected for Ornamental Use

Christos Nanos [1,†], Parthena Tsoulpha [2,†], Stefanos Kostas [3,*,†], Stefanos Hatzilazarou [3], Ioanna Michail [3], Vasiliki Anastasiadi [3], Elias Pipinis [4], Evangelos Gklavakis [5], Angelos K. Kanellis [6] and Irini Nianiou-Obeidat [1,*]

1 Laboratory of Genetics and Plant Breeding, School of Agriculture, Aristotle University, 54124 Thessaloniki, Greece; nanos95@hotmail.com
2 Laboratory of Forest Genetics and Plant Breeding, School of Forestry and Natural Environment, Aristotle University, 54124 Thessaloniki, Greece; thena@for.auth.gr
3 Laboratory of Floriculture, School of Agriculture, Aristotle University, 54124 Thessaloniki, Greece; hatzilaz@agro.auth.gr (S.H.); ioannamichail@hmu.gr (I.M.); vasakianas19@hotmail.com (V.A.)
4 Laboratory of Silviculture, School of Forestry and Natural Environment, Aristotle University, 54124 Thessaloniki, Greece; epipinis@for.auth.gr
5 Evangelos Gklavakis Nurseries, 58400 Aridea, Greece; evan.glavakis@gmail.com
6 Group of Biotechnology of Pharmaceutical Plants, Laboratory of Pharmacognosy, Department of Pharmaceutical Sciences, Aristotle University, 54124 Thessaloniki, Greece; kanellis@pharm.auth.gr
* Correspondence: skostas@agro.auth.gr (S.K.); nianiou@agro.auth.gr (I.N.-O.)
† These authors contributed equally to this work.

Abstract: *Salvia officinalis*, commonly known as sage, is highly valued for its medicinal and ornamental properties. In the present work, 12 native sage populations of north-west Greece were evaluated for eight ornamental traits. Populations from the locations of Aristi, Kefalovryso and Igoumenitsa were selected as the best performing and for their preservation and availability in the market, their asexual propagation was investigated by (a) shoot cutting and (b) in vitro techniques. Propagation by cuttings was investigated during the four seasons. Aristi exhibited the highest rooting (65%) in spring with a well-developed root system (4.7 root number and 5.0 cm length) by applying 0.5 g·L^{-1} Indole-3-butyric acid, potassium salt (K-IBA), established on perlite under a fog system. However, the rooting performance of Aristi spring cuttings was not affected by different substrates of peat:perlite (0:1, 1:1, 1:2 *v*/*v*) or rooting systems (mist, fog) tested. Furthermore, the in vitro propagation of the selected sage populations was investigated using shoot tips as explants. After successful disinfection, the effect of Murashige and Skoog (MS) medium in ten different combinations of Indole-3-acetic acid (IAA), 6-Benzylaminopurine (BAP) and Thidiazuron (TDZ) were tested on shoot multiplication. Aristi presented the highest number of newly formed shoots on MS9 (0.1 mg·L^{-1} IAA and 0.8 mg·L^{-1} TDZ) and MS5 (0.1 mg·L^{-1} IAA and 0.8 mg·L^{-1} BAP) (3.35 and 3.21 new shoots/explant, respectively) with the highest shoot length (2.23 cm and 3.2 cm) and unexpected spontaneous root formation (64%) at MS5. The rooting ability of Aristi microshoots was further investigated in order to enhance their response. Of the three rooting variants tested, optimal rooting formation (100%) was observed on 0.9 mg·L^{-1} IAA (R3) combined with successful acclimatization (100%). Aristi exceeded the other populations in both the tested propagation systems, thus holding a strong potential for its introduction in the market as a competitive ornamental variety.

Keywords: aesthetic use; cuttings; IBA; micropropagation; sage; season

check for updates

Citation: Nanos, C.; Tsoulpha, P.; Kostas, S.; Hatzilazarou, S.; Michail, I.; Anastasiadi, V.; Pipinis, E.; Gklavakis, E.; Kanellis, A.K.; Nianiou-Obeidat, I. Asexual Propagation of Greek *Salvia officinalis* L. Populations Selected for Ornamental Use. *Horticulturae* **2023**, *9*, 847. https://doi.org/10.3390/horticulturae9070847

Academic Editors: Konstantinos Bertsouklis, Epaminondas Kartsonas and Angela Carra

Received: 19 June 2023
Revised: 20 July 2023
Accepted: 21 July 2023
Published: 24 July 2023

1. Introduction

Salvia officinalis L., an evergreen, perennial species, is one of the most important of the genus *Salvia* and has been widely recognized for its medicinal, aromatic and culinary uses since ancient times [1–3]. The species is well known for its high content of essential

oils [4–8]. Moreover, due to the beautiful natural leaf, inflorescence variations and the species cold hardiness, *S. officinalis* is also an important ornamental plant [9].

The first step toward the improvement of a plant species by its desired traits is based on the analysis of the species morphology, mainly by assessing leaf, flower, fruit and plant shape variability [10], which is a useful tool for the selection of populations suitable as ornamentals [11,12]. However, the reliability of the evaluation by phenotypic characteristics is affected by environmental factors. The elimination of this effect is possible using a randomized experimental design and cultivating the plants in the same environment, field [12].

The need for readily available vegetative material directs research toward investigating and applying rapid and efficient propagating techniques, such as asexual propagation in vivo and in vitro.

For the successful rooting of sage cuttings, among the main factors investigated were the influence of season and the application of the most efficient indole-3-butyric acid (IBA) concentration [13–15]. Loconsole et al. [16] studied the effect of IBA dosage on the improvement of adventitious root quality on wild sage and some of its cultivars.

Concerning tissue culture, it is a useful, modern method of producing certified propagation material, for commercial use exploiting elite populations for their desired characteristics, having the advantage of retaining genetic stability and conserving plant genetic resources [17–19]. Especially for *S. officinalis*, several studies on its micropropagation using different explants have been conducted, such as from shoot tips [20,21], nodal segments [3,22,23], and axillary and apical buds [24]. Bolta et al. [25] investigated the cell cultures of the species. However, most of the previous works used in vitro propagation for the production of high value endogenous substances or for their bioactive molecules [17,21,25,26].

S. officinalis is a first-class alternative crop, with very good yields and a prominent position in the Greek market, holding the potential for further expansion. Although the majority of studies on sage focuses on the production of essential oil and other useful medicinal endogenous metabolites, there are no previous investigations of wild sage population morphological traits, selection or propagation of the best plant material for ornamental purposes.

The aim of this study was to evaluate the morphological traits of twelve sage populations from different regions of north-western Greece and select the best ones. For the populations of *S. officinalis* that stood out for their aesthetic morphology, asexual propagation by cuttings and in vitro techniques were studied in order to establish effective and functional propagation protocols.

2. Materials and Methods

2.1. Plant Material

Twelve wild-grown populations of *S. officinalis,* from different habitats of north-west Greece, were selected for evaluation. The code names and geographical coordinates of the central point of each population are given in Table 1. The sampling of plant material was conducted as follows: shoot cuttings were collected and transferred for propagation to the Evangelos Gklavakis nurseries (Piperia, Pella, Greece, latitude 40.964263 N, longitude 22.017363 E). The cuttings were treated with 0.5 g·L^{-1} Indole-3-butyric acid, potassium salt (K-IBA) (Sigma-Aldrich, St. Louis, MO, USA) and maintained under the fog system to root successfully. The young rooted plants were planted and grown for two years in an outdoor experimental collection of the same nursery (Table 1).

Shoots with leaves and flowers from the twelve populations were collected in June and transferred and kept in the herbarium (code numbers SO201-SO392) of the Floriculture Laboratory.

Table 1. Code names, coordinates of the central point of 12 *S. officinalis* populations and number of plants per population.

	Population	Latitude (North)	Longitude (East)	Number of Plants per Population
1	ARISTI	39.933689	20.679384	21
2	ARNISSA	40.798127	21.828355	9
3	ELAFOTOPOS	39.901731	20.692177	26
4	IGOUMENITSA	39.485731	20.264105	10
5	KEFALOVRYSO	40.003702	20.558476	11
6	KALPAKI	39.902897	20.641573	18
7	KALYBIA	39.902781	20.641872	15
8	KATO PEDINA	39.877418	20.670230	31
9	KERKYRA	39.770129	19.697890	10
10	MAYROBOUNI	39.954294	20.619267	18
11	MESOBOUNI	39.942593	20.646485	26
12	MIKROBALTOS	40.078276	21.872652	22

2.2. Analysis of Morphological Traits

The evaluated ornamental characteristics of the above plant material are analytically shown in Table 2. Traits No. 2–4 were measured in the laboratory with a ruler, and all the rest were measured directly at the outdoor experimental collection site (in June) [12].

Table 2. Morphological traits for the 12 selected populations of *S. officinalis* related to their ornamental value.

Morphological Traits	Description
1. Leaf number	Number of leaves per branch, 20 terminal branches (15 cm from the shoot tip) per population
2. Leaf length	In cm, measured from the base to the tip of adult /mature leaf, 50 leaves per population
3. Leaf width	In cm, measured at the widest part of adult leaf/mature, 50 leaves per population
4. Inflorescence length	In cm, measured from the base to the tip of inflorescence, 20 inflorescences per population
5. Node number per inflorescence	Number of nodes per inflorescence, 20 inflorescences per population
6. Flower number	Number of flowers per inflorescence, 20 inflorescences per population
7. Branch number	Number of terminal branches per plant, was measured in all plants
8. Branch length	Length of branches per plant, in cm, 20 terminal branches per population

2.3. Asexual Propagation of Selected S. officinalis Populations

Terminal shoots of the three best populations (Aristi, Igoumenitsa, Kefalovryso), selected for their ornamental traits, were harvested from the mother plants of the field collection in Piperia Aridea and used as starting material for both propagation techniques.

2.3.1. Propagation by Shoot Cuttings

Effect of K-IBA and Season on the Rooting of Cuttings

Terminal cuttings collected during the four seasons of 2021 were tested for their rooting ability. This type of cutting was reported as the most suitable for the propagation of *Salvia* [27]. The basal portion of each shoot cutting (8–10 cm, 5–6 leaves on the apical part) was dipped into aqueous solutions of 0, 0.5, or 1 g·L^{-1} of K-IBA for 10 s and planted in 10 L plastic trays (40 cm × 25 cm × 10 cm) filled with perlite (Isocom, Athens, Greece). The plastic trays were then established for rooting in a fog system, with the relative humidity (RH) adjusted to 95 ± 1%. Forty shoot cuttings were used for each treatment and population. After four weeks, the rooting ratio (%), as well as the number and length (cm) of roots, were recorded.

Effect of Substrate and the Mist or Fog System on Rooting of Cuttings

Terminal shoots of the best performing Aristi population were harvested in spring of the following year and used as cuttings. The effect of substrate was evaluated on three

different mixtures of peat TS2 Klasmann® (Klasmann-Deilmann, Geeste, Germany) and perlite: 0:1, 1:1, or 1:2 *v*/*v*, after treating the cuttings as previously described with 0.5 g·L^{-1} K-IBA. Finally, the cuttings were placed for rooting either under the fog or intermittent mist system. The RH in the fog system was adjusted to 95 ± 1%, while in the intermittent mist system, water was sprayed for 30 s every 30 min, from 06:00 to 22:00. In both rooting systems, the temperature at the bottom of the benches was set at 20 ± 1 °C using electrical cables. In each treatment, 40 shoot cuttings were used. Four weeks after planting, the rooting ratio (%) and the number and length (cm) of roots were measured.

2.3.2. In Vitro Propagation of *S. officinalis*

Explant Preparation and Disinfection

For the tissue culture experiments, shoot tips (2–3 cm) of the selected populations of *S. officinalis* were used as explants. First, they were pretreated with a mild dish soap and washed under running tap water for 20 min. Seven different disinfection treatments were tested for all populations (twenty explants each) (Table 3), followed by three successive washings with double distilled water (ddH_2O). The explants were established in vitro on MS [28] (Murashige and Skoog, 1962) medium, free of growth regulators, supplemented with sucrose (3%) and agar (0.8% Plant Agar, Duchefa Biochemie, The Netherlands). The pH was adjusted to 5.8. Cultures were maintained in plant growth chamber conditions: 23 ± 2 °C, 16 h photoperiod and light intensity at 50 µmol·m^{-2}·s^{-1} provided by cool-white fluorescent lamps. The same environmental conditions were applied to all subsequent experiments.

Table 3. Disinfection treatments applied to *S. officinalis* explants.

Treatment No.	(%) EtOH *v*/*v* *	Ascorbic and Citric Acid **	NaOCl (%)	Time Duration (min)
D1	70	–	0.06	10
D2	70	+	0.06	11
D3	70	–	0.06	12
D4	60	+	0.06	11
D5	50	+	0.06	12
D6	70	–	0.08	7
D7	70	–	0.04	17

* Stirring with ethanol solution for 30 s; ** A solution of ascorbic acid (300 mg·L^{-1}) and citric acid (200 mg·L^{-1}) for 15 min.

Effect of Growth Regulators on the Multiplication of *S. officinalis*

Clean explants were transferred on ten variants of MS nutrient media supplemented with different growth regulators: 6-Benzylaminopurine (BAP), Thidiazuron (TDZ) (Sigma-Aldrich, St. Louis, MO, USA) at concentrations of 0, 0.2, 0.5, 0.8 and 1.1 mg·L^{-1} combined with Indoleacetic acid (IAA) (0 or 0.1 mg·L^{-1}) (Sigma-Aldrich, St. Louis, MO, USA) (Table 4). A total of 600 shoots, 20 per population and substrate were used. The multiplication frequency, average number of shoots induced per explant and mean height of shoots were recorded after five weeks in culture.

In Vitro Rooting and Plantlet Acclimatization

Newly formed shoots (~3.5 cm) from the two best performing populations (Aristi, Kefalovryso originating from MS5 and MS9) were tested for rooting on MS supplemented with three variants of IAA: R1 (0.3 mg·L^{-1}), R2 (0.6 mg·L^{-1}), and R3 (0.9 mg·L^{-1} IAA). Fifteen microshoots per population and variant were used. For rooted plantlets with at least one root (>0.5 cm), the following were recorded: percentage (%) of rooted shoots and root length after five weeks. Rooted plantlets were transferred to acclimatization after thoroughly washing off the agar residue and established in propagating trays of 24 cells on TS-2 Klasmann® peat in a Styrofoam structure covered with plexiglass airtightly. The initial environmental conditions were as follows: 25 ± 2 °C, relative humidity 95 ± 1%,

12 h photoperiod (60 μmol·m^{-2}·s^{-1}) provided by artificial light. Gradually, in order to harden off the plants in a four-week period, the relative humidity was reduced to 65% ± 1 and the light intensity was adjusted to ambient (180 μmol·m^{-2}·s^{-1}). Plant survival was assessed after six weeks.

Table 4. Nutrient substrate composition for shoot multiplication.

Medium	IAA (mg·L^{-1})	BAP (mg·L^{-1})	TDZ (mg·L^{-1})
MS 1	0	0	0
MS 2	0.1	-	-
MS 3	0.1	0.2	-
MS 4	0.1	0.5	-
MS 5	0.1	0.8	-
MS 6	0.1	1.1	-
MS 7	0.1	-	0.2
MS 8	0.1	-	0.5
MS 9	0.1	-	0.8
MS 10	0.1	-	1.1

2.4. Statistical Analysis

For the morphological traits, the analysis of variance (ANOVA) was performed, while the separation of means was conducted by Duncan's multiple range test at $p \leq 0.05$. In all of the asexual propagation procedures, a complete randomized design was used. Four replicates of ten cuttings were used for the first asexual propagation technique, whereas three replications were used for each of the in vitro experiments. For in vitro and cuttings measurements, the mean ± standard deviation (SD) was calculated, while for morphological traits, the mean ± standard error (SE) was used. Mean comparisons were conducted using ANOVA and Duncan's test ($p \leq 0.05$). All percentages were subjected to arcsine transformation. Analyses were conducted using the SPSS V. 27 (IBM, Armonk, NY, USA) statistical package.

3. Results and Discussion

3.1. Morphological Analysis of Ornamental Traits

The results of the phenotypic evaluation indicated that the populations with the best ornamental properties were Aristi, Igoumenitsa, Kalpaki and Kefalovryso. According to the assessment, the trait "Leaf number" of the Kefalovryso population had the highest value, up to 30 leaves per branch and differed significantly from the others (Figure 1). Respectively, the lowest values were recorded for Kato Pedina and Kerkyra with less than seven leaves, although there was no statistical difference from all the other populations, apart from Kefalovryso, Arnissa, Igoumenitsa and Kalpaki. The populations Aristi and Kalpaki had the largest leaves concerning their "Leaf length", apart from Kato Pedina, which were statistically similar. In relation to "Leaf width", Igoumenitsa, Kerkyra and Arnissa had the widest leaves without being statistically different from most of the populations, whereas Kalybia was the narrowest. For "Inflorescence length", the lowest value was measured for Mikrobaltos (approximately 7.6 cm), followed by Kerkyra. In addition, the populations with the lowest values for the trait "Node number per inflorescence" were Kerkyra and Mikrobaltos. For the trait "Flower number", the lowest measurements were recorded for Arnissa, Mikrobaltos and Kerkyra. The highest values for the last two traits, "Branch Number and Length" per plant were observed for Igoumenitsa and Aristi, without statistical differences compared to the rest of the populations (Figure 1).

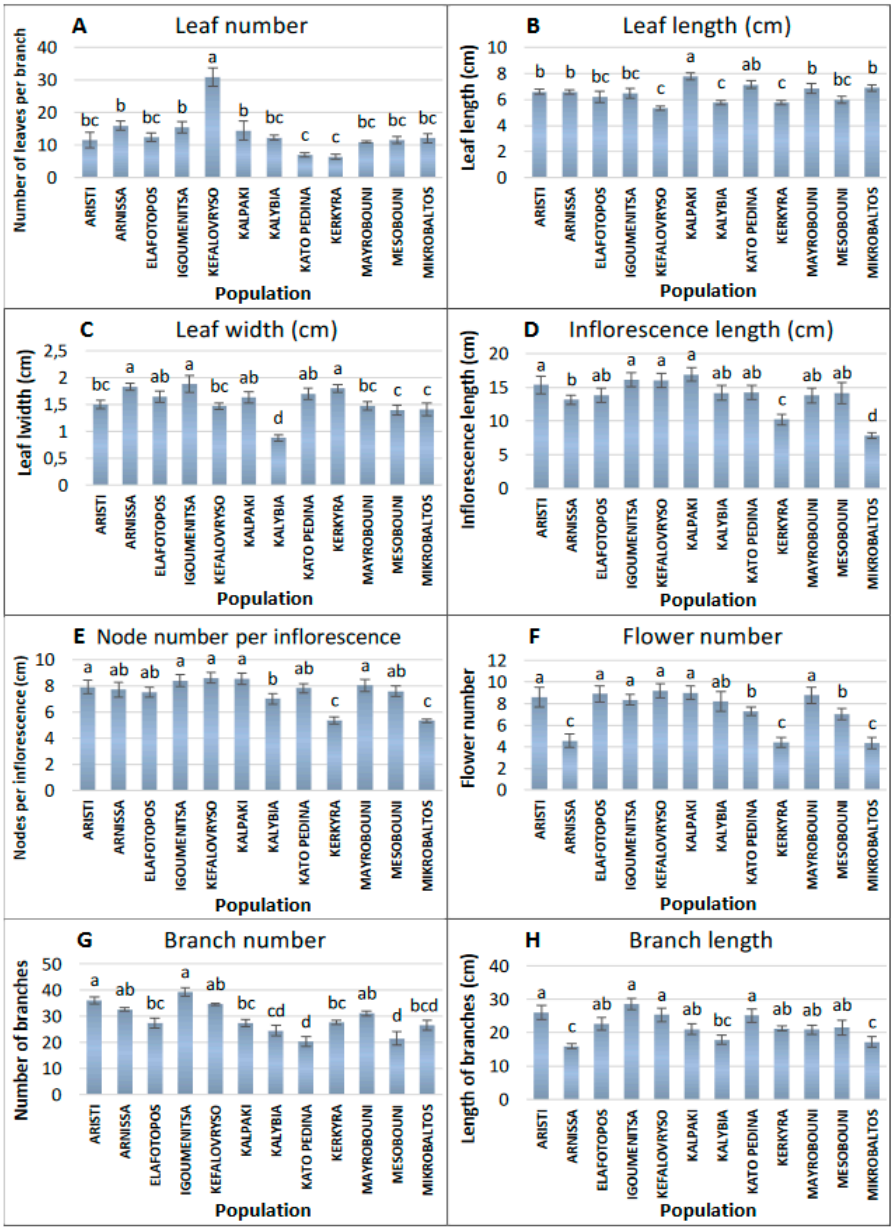

Figure 1. Mean values ± SE for eight morphological traits of ornamental interest [(**A**) Leaf number, (**B**) Leaf length (cm), (**C**) Leaf width (cm), (**D**) Inflorescence length (cm), (**E**) Node number per inflorescence, (**F**) Flower number, (**G**) Branch number and (**H**) Branch length] recorded from plants of the twelve populations of *S. officinalis* growing in an experimental field in Piperia (Pella, Greece). Different letters indicate statistically significant differences according to Duncan's multiple range test at $p \leq 0.05$, error bars indicate standard errors.

In general, the evaluation of morphological data revealed significant phenotypic variability among the twelve populations of *S. officinalis.* More specifically, Igoumenitsa was the population with the better morphological traits, while Kefalovryso presented better values not only in the number of nodes and flowers per inflorescence but also in the number of leaves per branch. However, better values in both leaf and inflorescence length were recorded for the Aristi population. According to the above, the Igoumenitsa, Kefalovryso and Aristi populations were selected as the most suitable for decorative use and their asexual propagation was further investigated by tissue culture and shoot cutting techniques.

Thus far, there has been limited research on the morphological characterization of *S. officinalis* for ornamental purposes. However, several studies have been conducted to estimate the phenotypic diversity of different species of *Salvia* using both qualitative and quantitative morphological traits [29–31]. Leontaritou et al. [32] evaluated the morphological diversity (leaf and floral traits) of 49 individuals from *Salvia pomifera* subsp. *calycina* (Sm.) Hayek (Apple sage), originating from five natural populations of the Peloponnese (Greece). In that study, leaf length ranged from 3.88 to 5.23 cm, while in the present, it ranged from 5.3 to 7.8 cm. Concerning leaf width and inflorescence length, the range of values was higher than in our recorded measurements. These could be attributed to variations between *Salvia* species [32].

In another study, the morphological traits of *Salvia fruticosa* (Greek sage) were also evaluated. Ten populations of *S. fruticosa,* from different locations of the Peloponnese, were evaluated and found to differ significantly for both leaf and floral traits. The highest value of leaf length was 4.32 cm instead of 7.8 cm in our study, while the leaf width presented the same size. The researchers concluded that this morphologic variability could be attributed to environmental parameters, such as altitude, latitude and climatic type [33].

Similar studies on the morphological traits of other plants of the Lamiaceae family, such as *R. officinalis,* indicated a significant phenotypic variability among the seven rosemary populations tested [12]. Using 15 qualitative traits, Zigene et al. [34] recorded the phenotypic diversity of 45 Ethiopian rosemary accessions from different growing regions. Morphological traits were also used in *Mentha longifolia* to describe a significant positive correlation between morphological and phytochemical characteristics [35]. Furthermore, the phenotypic and genetic diversity among 19 different populations of *Mentha longifolia* from various altitudes were also examined [36].

In general, a significant amount of phenotypic diversity exists in morphological traits among populations of the Lamiaceae plant species, which could be used to distinguish accessions of different growth regions for future selection and characterization work for various uses [34].

3.2. Asexual Propagation of Selected S. officinalis Populations

3.2.1. Propagation by Shoot Cuttings

Effect of K-IBA and Season on Cutting Rootability

The season of cutting collection, as well as the application of K-IBA, influenced the rooting of the three selected populations of *S. officinalis* (Figure 2). Spring proved to be the best season for rooting for all studied populations, with figures up to 65% (Aristi treated with 0.5 g·L^{-1} K-IBA), while no rooting was noticed in summer, except for Aristi cuttings treated with 1 g·L^{-1} K-IBA (10% rooting). In autumn and winter, rooting percentages reached up to 30 and 15%, respectively, for Aristi. Season influences the rooting ability of shoot cuttings due mainly to the different physiological status and the different lignification levels of the tissues among the four seasons. As Nikola et al. [13] reported, the best period for rooting of *S. officinalis* shoot cuttings was from spring to the end of autumn. These results are partly in agreement with our findings that the best season for rooting sage shoot cuttings was spring.

The application of K-IBA, regardless of concentration, significantly increased the rooting rate, even up to five-fold, as compared with the control. The concentration of 0.5 g·L^{-1} K-IBA was more effective on rooting than 1 g·L^{-1} in the spring, autumn and winter collection of cuttings for Aristi, whereas it was less effective in summer and winter for Kefalovryso and in spring for Igoumenitsa. In all other cases, both concentrations of K-IBA were similarly effective in rooting (Figures 2 and 3).

Thus, the application of K-IBA (0.5 g·L^{-1}) in spring increased the rooting of Aristi from 12.5 to 65%, while in the Kefalovryso population from 10% (control) to 47%. For the Igoumenitsa population, rooting reached 22.5% only in the presence of 1 g·L^{-1} K-IBA.

The number and length of the roots of *S. officinalis* cuttings were influenced by both studied factors. In particular, the number of roots increased significantly in the presence

of K-IBA compared to the control, especially its higher concentration (1 g·L^{-1}) (Table 5). The highest number of roots was recorded in Aristi during spring and autumn, with 1 g·L^{-1} K-IBA (5.3 and 5.4 roots per cutting, respectively) (Table 5). The populations of Igoumenitsa and Kefalovryso also formed many roots on the same auxin level during winter, spring and autumn (4.4, 4.7, 4.8 and 4.2, 4.4, 4.2 roots per cutting, respectively) (Table 5). Concerning the length of roots, the control showed the best response for Aristi in spring and autumn (5.9 and 5.5 cm, respectively) but for Kefalovryso, only in spring (5.8 cm) (Table 5). For Igoumenitsa, the best result was observed during spring on 0.5 g·L^{-1} K-IBA (5.3 cm), followed by autumn on 1 g·L^{-1} K-IBA (4.8 cm) (Table 5).

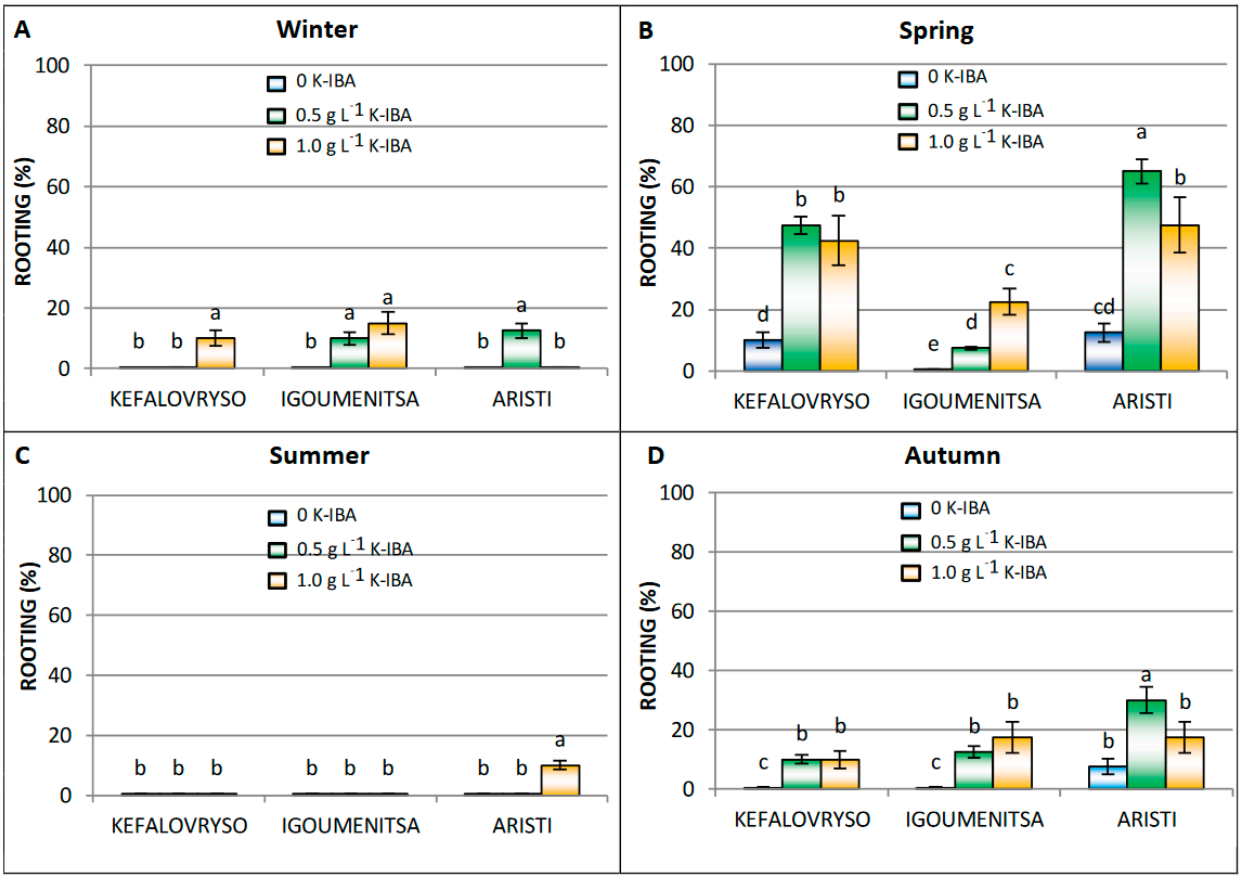

Figure 2. Effect of season, (**A**) winter, (**B**) spring, (**C**) summer, (**D**) autumn, and K-IBA on rooting (%) of *S. officinalis* Kefalovryso, Igoumenitsa and Aristi shoot cuttings (±standard deviation). Different letters indicate statistical differences, according to Duncan's multiple range test ($p \leq 0.05$).

All previous studies on sage cuttings reported the enhancement of rooting ratio and quality of the rooting system in the presence of auxin. For the successful rooting of sage cuttings, IBA was mainly used. Ayanoglu and Ozkan [37] reported that the application of 100 ppm IBA of sage cuttings led to quick rooting formation (78.75%) on the 15th day from establishment. Kara et al. [14] reported 81% rooting by applying 4000 ppm IBA and a well-developed rooting system (10.6 roots per cutting, 5.1 cm in length). Paradikovic et al. [15] achieved optimal rooting (100%) by applying Rhizopon powder (0.5% *w*/*w* IBA) on green cuttings of *S. officinalis.* Loconsole et al. [16] found that rooting system quality of the wild sage cultivar 'Little Lucky' was improved by 5000 mg·L^{-1} IBA, but it was less effective for 'Yellow'.

The improvement in the quality of the rooting system of sage cutting with the application of auxin agrees with the results of the present study. Finally, the results of our study, which confirm that the sensitivity to IBA dosage varies among species and their

cultivars [38], could be relevant to the production of high-quality cuttings in the commercial nursery industry.

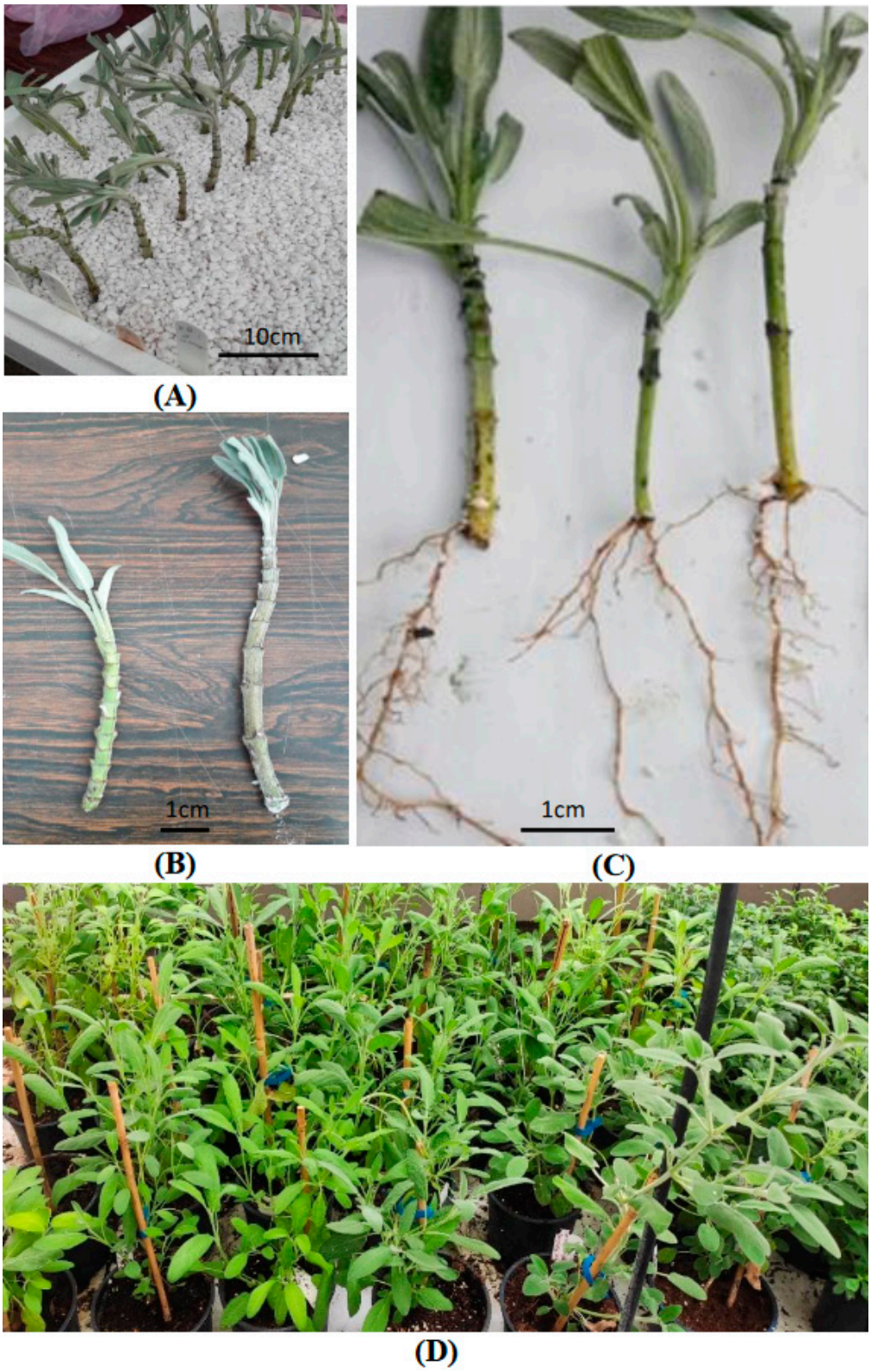

Figure 3. (**A**) Shoot cuttings of *S. officinalis*, (**B**) Planted cuttings of *S. officinalis* in perlite, in a fog system for rooting, (**C**) Rooted shoot cuttings of *S. officinalis* Kefalovryso, Igoumenitsa and Aristi (from left to right) treated with 0.5 g·L^{-1} K-IBA during spring and (**D**) *S. officinalis* plants from the population Aristi growing in greenhouse, six months after rooting of shoot cuttings.

Table 5. Effect of season (winter, spring, summer and autumn) and K-IBA on number and length (cm) of roots of *S. officinalis* Kefalovryso, Igoumenitsa and Aristi shoot cuttings.

	K-IBA g·L^{-1}	Number of Roots				Length of Roots (cm)			
		Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Kefalovryso	0	-	$2.5 \pm 0.3^{d,*}$	-	-	-	5.8 ± 0.5^{a}	-	-
	0.5	-	$3.8 \pm 0.1^{b,c,**}$	-	3.4 ± 0.3^{c}	-	5.1 ± 0.2^{b}	-	$4.8 \pm 0.2^{b,c}$
	1.0	$4.2 \pm 0.2^{a,b}$	4.4 ± 0.3^{a}	-	$4.2 \pm 0.3^{a,b}$	4.2 ± 0.4^{d}	$4.4 \pm 0.4^{c,d}$	-	$4.6 \pm 0.3^{b,c}$
Igoumenitsa	0	-	-	-	-	-	-	-	-
	0.5	3.8 ± 0.3^{b}	3.9 ± 0.3^{b}	-	$4.3 \pm 0.2^{a,b}$	$4.6 \pm 0.2^{b,c}$	5.3 ± 0.2^{a}	-	$4.7 \pm 0.4^{a,b}$
	1.0	4.4 ± 0.1^{a}	4.7 ± 0.2^{a}	-	4.8 ± 0.4^{a}	4.1 ± 0.4^{c}	$4.8 \pm 0.3^{a,b}$	-	$4.8 \pm 0.3^{a,b}$
Aristi	0	-	3.1 ± 0.1^{c}	-	2.9 ± 0.1^{c}	-	5.9 ± 0.4^{a}	-	$5.5 \pm 0.4^{a,b}$
	0.5	4.7 ± 0.3^{b}	4.7 ± 0.2^{b}	-	4.6 ± 0.4^{b}	$4.7 \pm 0.3^{b,c}$	5.0 ± 0.2^{b}	-	$4.7 \pm 0.5^{b,c}$
	1.0	-	5.3 ± 0.2^{a}	2.2 ± 0.1^{d}	5.4 ± 0.2^{a}	-	4.9 ± 0.3^{b}	3.4 ± 0.2^{d}	4.2 ± 0.3^{c}

* Standard Deviation; ** Means in each population, for number and length of roots, with different letters indicate statistically significant differences, according to Duncan's multiple range test ($p \leq 0.05$).

Effect of Substrate and Mist or Fog Systems on the Rooting of Cuttings

The rooting of shoot cuttings collected from Aristi plants was affected by both the type of substrate and the kind of rooting system (Figure 4). The highest rooting of cuttings was observed on substrates of 1:0 and 2:1 perlite and peat in the fog system (60% and 37.5%, respectively) (Figure 4). No rooting was observed when a mixture of perlite and peat (1:1 v/v) was used as a substrate.

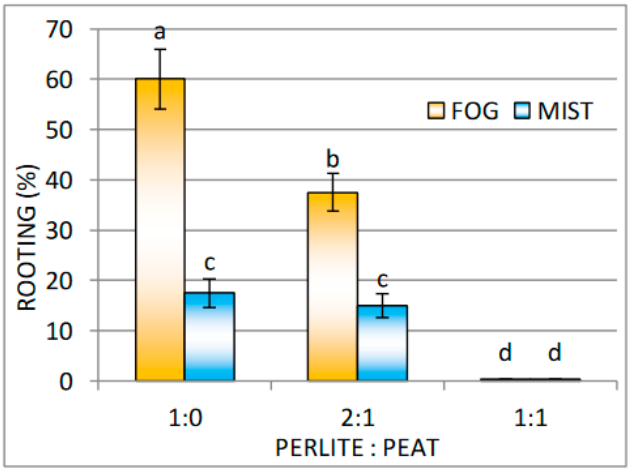

Figure 4. Effect of rooting system (fog or mist) and substrate composition (perlite:peat 1:0, 2:1 and 1:1) on rooting (%) of *S. officinalis* shoot cuttings (±Standard deviation) of population Aristi, in spring. Different letters indicate statistically significant differences, according to Duncan's multiple range test ($p \leq 0.05$).

The fog system increased the rooting ratio of the cuttings more than three-fold in the perlite: peat (1:0 v/v) substrate and more than two-fold in the perlite: peat (2:1 v/v) substrate compared with the mist system (Figure 4). The number and length of the new roots did not show any significant differences among the two tested substrates for both fog and intermittent mist systems (Table 6). Thus, 3–4 new roots 3.5–4.4 cm in length were formed (Table 6). In general, shoot cuttings of *S. officinalis* were rooted adequately on the substrate of perlite and peat (1:0, v/v) in the fog rooting system (Figure 4).

Other researchers studying the rooting of sage stem cuttings have also found differences in rooting in relation to the substrate tested [39,40]. In agreement with our results, Vârban et al. [41] concluded that perlite was the most appropriate substrate for the rooting of *S. officinalis* cuttings.

The selected sage populations were also propagated using the in vitro technique in order to optimize rooting results.

Table 6. Effect of rooting system (fog or mist) and substrate composition (perlite:peat 1:0, 2:1 and 1:1) on number and length (cm) of roots of *S. officinalis* Aristi shoot cuttings in spring.

Perlite:Peat	Fog		Mist	
	Number of Roots	Length of Roots (cm)	Number of Roots	Length of Roots (cm)
1:0	4.2 ± 0.3 [a,*,**]	3.9 ± 0.4 [a]	3.7 ± 0.7 [a]	4.0 ± 0.5 [a]
2:1	4.8 ± 0.6 [a]	4.7 ± 0.5 [a]	4.3 ± 0.5 [a]	3.8 ± 0.4 [a]
1:1	-	-	-	-

* Standard deviation; ** Different letters in the same column indicate statistically significant differences according to Duncan's multiple range test ($p \leq 0.05$).

3.2.2. In Vitro Propagation of *S. officinalis*

Effect of Disinfection Treatments on *S. officinalis*

Of the seven disinfection treatments tested, four of them (D1-D4), were equally successful for all three populations (Table 7). However, due to phenol presence in the medium, explant necrosis was observed except for the second treatment (D2), where an antioxidant solution (300 mg·L^{-1} ascorbic acid and 200 mg·L^{-1} citric acid) was applied, which provided healthy and vibrant explants (Figure 5A). Thus, it was chosen as the most appropriate for the surface sterilization of the studied sage populations: 95% for Aristi, 85% for Kefalovryso and 75% for Igoumenitsa, without necrosis.

Table 7. Survival percentage of explants of three *S. officinalis* populations.

Treatment	Percentage of Survival		
	Aristi	Kefalovryso	Igoumenitsa
D1	80.0 ± 9 [a,b,*,**]	75.0 ± 9 [a]	65.0 ± 10 [a]
D2	95.0 ± 5 [a]	85.0 ± 8 [a]	75.0 ± 9 [a]
D3	80.0 ± 8 [a,b]	65.0 ± 10 [a,b]	60.0 ± 11 [a]
D4	65.0 ± 10 [b]	60.0 ± 11 [b]	65.0 ± 4 [a]
D5	45.0 ± 11 [c]	30.0 ± 10 [c]	35.0 ± 10 [b]
D6	30.0 ± 10 [c]	20.0 ± 11 [c]	25.0 ± 9 [b]
D7	40.0 ± 11 [c]	40.0 ± 11 [c]	35.0 ± 10 [b]

* Standard deviation; ** Different letters in the same column indicate statistically significant differences according to Duncan's multiple range tests at $p \leq 0.05$.

Other researchers succeeded in obtaining clean explants of *S. officinalis* using a disinfection treatment similar to that of the present work. Bolta et al. [25] succeeded in disinfecting young shoots in ethanol solution (70% *v*/*v*) and NaOCl (0.5% *w*/*v*). In other works, the same procedure was followed, but either 1% NaOCl was used with a few drops of Tween-02 [42] or the explants were immersed in a 0.1% $HgCl_2$ (Mercury II chloride) solution [43]. According to the literature, a variety of disinfectants were used in other members of Lamiaceae, with the most common being commercial sodium hypochlorite (NaOCl), EtOH and mercury chloride ($HgCl_2$) solutions [26,44].

In the present work, the problem of explant browning and necrosis noticed at this stage was eliminated by immersing plant material in an antioxidant solution (ascorbic acid and citric acid). The same practice was successfully followed for another member of the Lamiaceae family, *Rosmarinus officinalis* [11] and one of the Rosaceae, *Pyrus spinosa* [45]. It is known that ascorbic acid prevents the browning and hyperhydricity of explants and improves in vitro rooting and ex vitro survival of the plants [17,46,47].

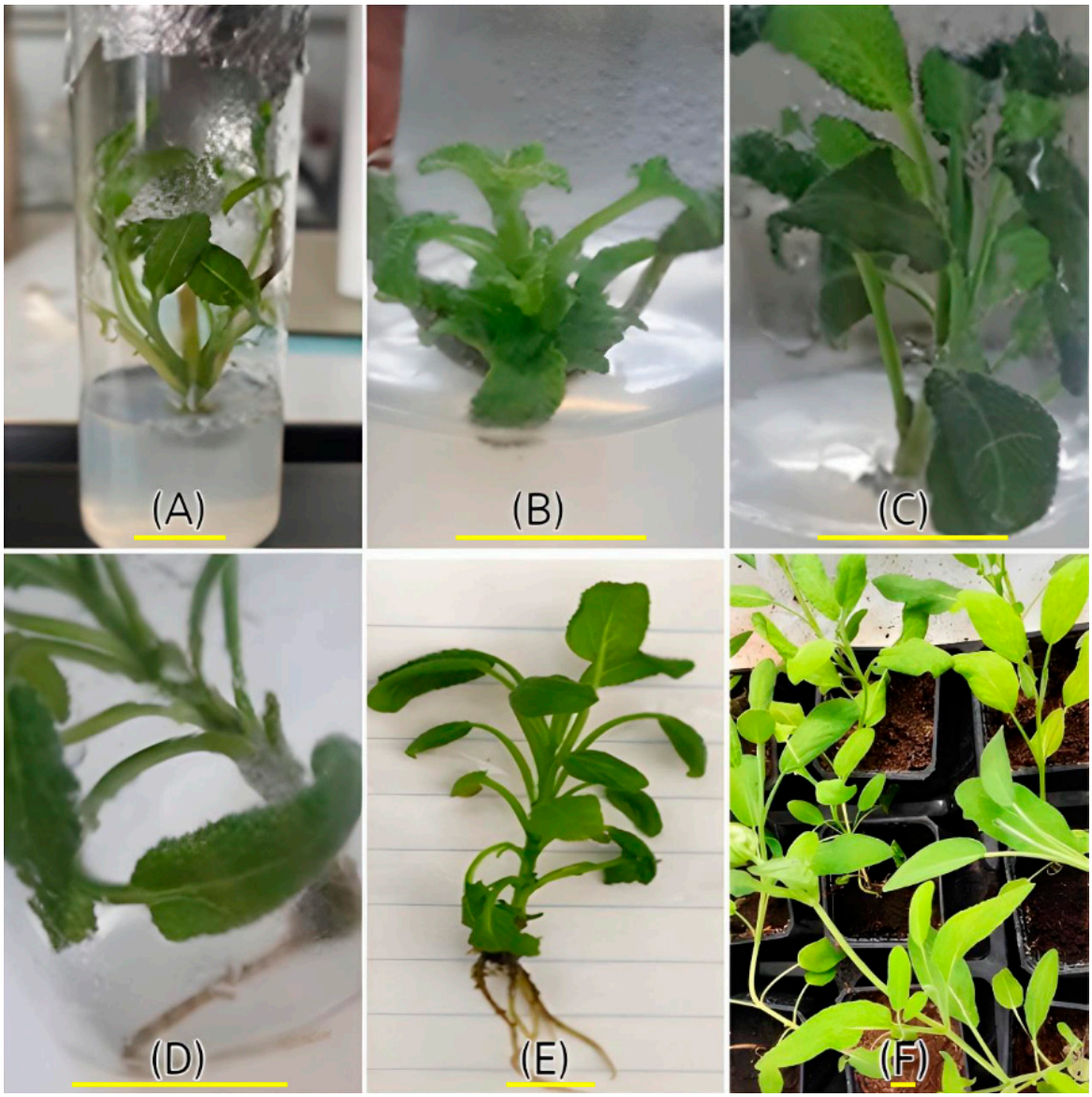

Figure 5. In vitro propagation of *Salvia officinalis* Aristi (**A**–**C**) multiplication on MS5 medium, (**D**,**E**) rooting ability on medium R3 and (**F**) acclimatization of plants in greenhouse. The yellow bars represent the size of 1 cm.

Effect of Growth Regulators on the Propagation of *S. officinalis*

Ten MS media, enriched with different combinations and concentrations of IAA with BAP or TDZ, were studied for Aristi, Kefalovryso and Igoumenitsa. Nutrient media MS5 and MS9 proved the best for the multiplication of explants (Tables 8 and S1, Figure S1).

In particular, Aristi exhibited the largest number of shoots on MS9 and MS5 media (3.35 and 3.21 new shoots/explant, respectively), significantly different from all other treatments (Figure 5A–C). Additionally, shoot length was the highest on the same media, i.e., 2.23 cm and 3.2 cm, respectively. For the same treatments, Kefalovryso was the second best for shoot production (2.4 and 2.3 new shoots/ explant, respectively) and shoot length (1.94 and 2.77 cm, respectively). Igoumenitsa exhibited the lowest number of new shoots on MS1 and MS7, which statistically differed from all other studied media. Concerning shoot length, it reached 2.17 cm on MS5 (Figure S2, Table S2).

Table 8. Effect of plant growth regulators on multiplication of *S. officinalis* populations.

Nutrient Medium	Number of Shoots			Length of Shoots (cm)		
	Aristi	Kefalovryso	Igoumenitsa	Aristi	Kefalovryso	Igoumenitsa
MS1	1.28 ± 0.43 ^{b,*,**}	0.57 ± 0.25 ^{c}	0.20 ± 0.16 ^{b}	0.28 ± 0.09 ^{c}	0.27 ± 0.15 ^{d}	0.42 ± 0.22 ^{b}
MS2	1.21 ± 0.36 ^{b}	0.78 ± 0.31 ^{c}	0.50 ± 0.25 ^{a,b}	0.38 ± 0.21 ^{c}	0.72 ± 0.33 ^{c,d}	0.44 ± 0.22 ^{b}
MS3	1.42 ± 0.44 ^{b}	1.07 ± 0.32 ^{b,c}	0.42 ± 0.25 ^{a,b}	0.53 ± 0.21 ^{c}	0.88 ± 0.35 ^{c,d}	0.90 ± 0.47 ^{a,b}
MS4	1.64 ± 0.42 ^{b}	1.14 ± 0.37 ^{b,c}	0.57 ± 0.30 ^{a,b}	1.25 ± 0.38 ^{b,c}	1.17 ± 0.46 ^{b,c}	1.10 ± 0.59 ^{a,b}
MS5	3.21 ± 0.40 ^{a}	2.30 ± 0.43 ^{a}	1.00 ± 0.37 ^{a}	3.20 ± 0.50 ^{a}	2.77 ± 0.54 ^{a}	2.17 ± 0.80 ^{a}
MS6	1.21 ± 0.48 ^{b}	2.00 ± 0.49 ^{a,b}	0.57 ± 0.27 ^{a,b}	0.95 ± 0.39 ^{b,c}	0.87 ± 0.34 ^{c,d}	1.17 ± 0.52 ^{a,b}
MS7	0.78 ± 0.28 ^{b}	0.70 ± 0.30 ^{c}	0.28 ± 0.19 ^{b}	1.50 ± 0.46 ^{b,c}	0.97 ± 0.38 ^{c,d}	0.42 ± 0.28 ^{b}
MS8	1.78 ± 0.49 ^{b}	1.07 ± 0.33 ^{b,c}	0.57 ± 0.30 ^{a,b}	1.39 ± 0.49 ^{b,c}	1.25 ± 0.46 ^{b,c}	0.77 ± 0.41 ^{b}
MS9	3.35 ± 0.55 ^{a}	2.40 ± 0.40 ^{a}	0.92 ± 0.40 ^{a}	2.23 ± 0.50 ^{a,b}	1.94 ± 0.47 ^{a}	1.50 ± 0.65 ^{a,b}
MS10	1.28 ± 0.39 ^{b}	1.07 ± 0.33 ^{b,c}	0.50 ± 0.27 ^{a,b}	1.49 ± 0.50 ^{b,c}	1.60 ± 0.44 ^{a,b}	1.07 ± 0.56 ^{a,b}
Average	1.71 ± 0.87	1.31 ± 0.67	0.55 ± 0.25	1.32 ± 0.89	1.24 ± 0.71	0.99 ± 0.55

* Standard deviation; ** Different letters in the same column indicate statistically significant differences according to Duncan's multiple range test at $p \leq 0.05$.

According to the bibliography, the search for an ideal combination of plant growth regulator concentrations has been extensively studied to obtain the best possible result in explant propagation. Cytokinins play the most important role in shoot development and auxins in rooting [48]. Several reports comment on the best combination of growth regulators for the multiplication of *S. officinalis*. Mohamed et al. [49] studied the effect of plant growth regulators on organogenesis of *S. officinalis* on nodal explants using MS medium with 0.1 mg·L^{-1} IAA and 1.5 mg·L^{-1} TDZ and reported the highest shoot production (7.2 shoots/explant) and shoot length (3 cm). Gostin [3] found that MS with 2.22 µM BAP was the best treatment for multiplication rate (100%) with length ranging from 4.03 to 4.59 cm in all media that contained BAP. The most relevant to our work is that of Grzegorczyk et al. [20,21], who reported that after three weeks from seed establishment in vitro, an average of 3 shoots per explant emerged on MS with 0.1 mg·L^{-1} IAA and 0.45 mg·L^{-1} BA. The results of the above-mentioned works agree with the findings of the present study.

In related research of other *Salvia* species, BA and BAP were the most frequently used cytokinins: Kintzios et al. [4] for the somatic embryogenesis of *S. officinalis,* for the multiplication *S. blancoana and S. valentine* [50], for *S. fruticosa* [51], while for *S. elegans*, *S. sinaloensis*, *S. cinnabarina* and *S. jamensis* Mascarello et al. [52] used low levels of BA. The same cytokinins have also been effectively used for the micropropagation of various plant species from the Lamiaceae family with economic interest [53]. For *Rosmarinus officinalis* adventitious shoot formation, the optimum level was 5 mg·L^{-1} 6-BAP [44]. For the same species, the highest shoot frequency was achieved on MS without growth regulators or in combinations of BAP (0.25 or 0.5 mg·L^{-1}) and IAA (0.1 mg·L^{-1}) [12]. Mehalaine and Chenchouni [26] showed that the combinations of IAA and Kin exhibited significant effects on callus and shoot proliferation in *T. algeriensis*, *R. officinalis* and *M. vulgare* in in vitro micropropagation. The culture medium is one of the most critical factors contributing to successful micropropagation [44].

Spontaneous Rooting of *S. officinalis* Explants

After the fifth week of establishment on the multiplication stage, an unexpected result was observed, i.e., the spontaneous root formation on five media of Aristi and Kefalovryso populations and on four of Igoumenitsa (Tables 9 and S3, Figure S3). The nutrient medium MS5 (0.1 mg·L^{-1} IAA and 0.8 mg·L^{-1} BAP) resulted in the highest rooting rates for Aristi (64%), Kefalovryso (57%) and Igoumenitsa (28%), the last of which, however, does not differ from MS9 (0.1 mg·L^{-1} IAA and 0.8 mg·L^{-1} TDZ) (14%) (Table 9). On the same medium (MS5), explants of two populations also exhibited the largest length of roots: for Aristi, 4.01 cm with root hairs, and for Kefalovryso, 3.03 cm. Igoumenitsa showed the lowest root length compared to the other two populations, with the best response at 1.92 cm on

MS9. Comparing the three populations, Aristi showed the best response concerning root percentage and root length. The microshoots that rooted spontaneously were immediately transferred to acclimatization.

Table 9. Effect of plant growth regulators on spontaneous rooting of *S. officinalis* populations.

Nutrient Medium	Rooting Formation (%)			Length of Roots (cm)		
	Aristi	Kefalovryso	Igoumenitsa	Aristi	Kefalovryso	Igoumenitsa
MS1	0^{b}	0^{b}	0^{b}	-	-	-
MS2	0^{b}	0^{b}	0^{b}	-	-	-
MS3	0^{b}	0^{b}	0^{b}	-	-	-
MS4	$7 \pm 7^{b,*,**}$	7 ± 7^{b}	0^{b}	0.94 ± 0.21^{c}	0.91 ± 0.19^{c}	-
MS5	64 ± 13^{a}	57 ± 13^{a}	28 ± 12^{a}	4.01 ± 0.51^{a}	3.03 ± 0.40^{a}	1.00 ± 0.18^{b}
MS6	7 ± 7^{b}	7 ± 7^{b}	7 ± 7^{b}	1.21 ± 0.19^{c}	1.00 ± 0.17^{c}	0.57 ± 0.13^{c}
MS7	0^{b}	0^{b}	0^{b}	-	-	-
MS8	0^{b}	0^{b}	0^{b}	-	-	-
MS9	21 ± 11^{b}	14 ± 9^{b}	$14 \pm 9^{a,b}$	3.05 ± 0.41^{b}	2.02 ± 0.35^{b}	1.92 ± 0.30^{a}
MS10	7 ± 7^{b}	7 ± 7^{b}	7 ± 7^{b}	1.01 ± 0.23^{c}	0.90 ± 0.20^{c}	$0.85 \pm 0.18^{b,c}$
Average	10.6 ± 19.8	9.2 ± 17.4	5.6 ± 9.2	2.32 ± 1.45	1.73 ± 0.99	1.08 ± 0.58

* Standard deviation, ** Different letters in the same column indicate statistically significant differences according to Duncan's multiple range test at $p \leq 0.05$.

These findings are in complete accordance with those of Petrova et al. [17]. The aim of the study was to develop an efficient method for micropropagation of *S. officinalis*, as well as to evaluate flavonoid content and antioxidant capacity in leaves of the obtained shoots. At the multiplication stage, using nodal segments from in vitro seedlings established on MS with 0.5 mg·L^{-1} BAP and 0.1 mg·L^{-1} IAA, a rooting percentage of 40% was recorded, while on MS with 0.5 mg·L^{-1} Zeatin and 0.1 mg·L^{-1} IAA, even higher rooting was observed (75%). In a similar combination of growth regulators (0.8 mg·L^{-1} BAP and 0.1 mg·L^{-1} IAA), at the same stage, in the present work, we also obtained spontaneous rooting of all populations, with Aristi performing the best (64%). Another species of Lamiaceae, *Lavandula pedunculata*, also exhibited the best propagation rates and spontaneous rooting in MS with 0.10 mg·L^{-1} BA [54]. In a study on *S. officinalis*, Gostin [3] observed no rooting induction on a substrate with IBA. For the same species, Ioja-Boldura et al. [43] tested rooting on MS with the presence or absence of 4.92 µM IBA. In the case of MS-free medium, rooting reached 97% within two weeks, while in the presence of IBA, microshoots rooted 48% after one month. The above observations verify our results of spontaneous rooting, which is probably due to high levels of endogenous auxins in *S. officinalis* tissues, which enables rooting without applying auxins exogenously.

In Vitro Rooting and Plantlet Acclimatization

The rooting ability of microshoots of the two best performing populations in shoot multiplication was further investigated in order to elevate percentages. More specifically, microshoots from Aristi (Figure 5A–C) and Kefalovryso populations originating from MS5 and MS9 were transferred on three different MS rooting variants with IAA: R1, R2 and R3 (Tables 10 and S4, Figure S4). Optimal rooting (100%) was recorded on R3 medium (0.9 mg·L^{-1} IAA) after seven weeks for Aristi grown on MS5, while from MS9 reached 66.7% (Figure 5D,E). For Kefalovryso, the respective rooting percentages were 66.7% and 41.6%.

Both populations from all treatments showed optimal results in acclimatization, either from spontaneous or rooting experiments. In particular, both Aristi and Kefalovryso exhibited equally excellent survival rates (~88 to 100%) (Table 10). Acclimatized plants were transferred to greenhouse conditions where vibrant growth and healthy sage plants continued to grow after one month (Figure 5F).

Table 10. Rooting and acclimatization of *S. officinalis* per population and propagation medium.

Population	Propagation Medium	Rooting Medium	Rooting (%)	Acclimatization (%)
Aristi	MS5	R1	$16.6 \pm 11^{d,*,**}$	100^{a}
	MS5	R2	$33.3 \pm 14^{b,c,d}$	100^{a}
	MS5	R3	100^{a}	92.0 ± 5^{a}
	MS5	- ***	64.0 ± 13^{b}	88.8 ± 7^{a}
	MS9	R1	$41.6 \pm 14^{b,c}$	100^{a}
	MS9	R2	$41.6 \pm 14^{b,c}$	100^{a}
	MS9	R3	66.7 ± 14^{b}	100^{a}
	MS9	-	$21.0 \pm 11^{c,d}$	100^{a}
Kefalovryso	MS5	R1	$33.3 \pm 14^{b,c,d}$	100^{a}
	MS5	R2	$33.3 \pm 14^{b,c,d}$	100^{a}
	MS5	R3	66.7 ± 14^{b}	87.5 ± 6^{a}
	MS5	-	57.0 ± 13^{b}	100^{a}
	MS9	R1	$25 \pm 13^{c,d}$	100^{a}
	MS9	R2	$41.6 \pm 14^{b,c}$	100^{a}
	MS9	R3	$41.6 \pm 14^{b,c}$	100^{a}
	MS9	-	14.0 ± 9^{d}	100^{a}

* Standard deviation, ** Different letters in the same column indicate statistically significant differences according to Duncan's multiple range test at $p \leq 0.05$, *** i.e., spontaneous rooting.

Ghanbar et al. [55], aiming at the in vitro bud induction and shoot regeneration of *Salvia sclarea*, observed that MS in combination with IAA (0.5 mg·L^{-1}) reached the highest levels of rooting (87–100%) and acclimatization 90%, results similar to those of the present study. For *Salvia officinalis*, Jafari et al. [56] observed a 72% rooting rate and 3.9 root number on MS with IBA (1 mg·L^{-1}) after 45 days. Arikat et al. [51], for the micropropagation and accumulation of essential oils in *Salvia fruticosa*, reported a high rate of rooting (90%) by adding 2.7 μM IBA. In addition, Gostin [3] observed that the addition of kinetin (4.65 μM) promoted rooting in contrast to the effect of the same cytokinin with NAA (2.68 μM). The above results indicate the important role of the initial propagation medium in the optimization of rooting response and support the observations of spontaneous rooting and excised conclusions on rich endogenous auxin background of *S. officinalis* populations.

The propagating medium might play a key role by using it alternatively in two ways: (a) as a medium for multiplication and spontaneous rooting and (b) providing the appropriate vegetative material for the subsequent rooting stage. Acclimatization was successful for both the populations for material originating either from spontaneous or in vitro rooted plant material.

4. Conclusions

The present study will contribute to the sustainable exploitation and promotion of selected native sage populations for decorative uses with simultaneous conservation of this valuable genetic material and the provision of the market with the required vegetative plant material by either cuttings or in vitro techniques. Of the three selected wild sage populations, the results proved that both techniques adequately justified the main goals, i.e., selection and asexual propagation of the present research. Even though rooting of cuttings reached satisfactory results, in vitro propagation enhanced to the optimal the rooting and acclimatization of the selected populations. Overall, the population of Aristi has the dynamics of a new ornamental sage variety, and as such, it can be introduced in the market of plants with aesthetic value.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9070847/s1, Figure S1: Effect of medium and population and their interactions on in vitro shoot regeneration, Figure S2: Effect of growth regulators on the length of newly formed shoots per population, Figure S3: Spontaneous rooting (%) on multiplication media per population, Figure S4: Comparison of the two populations Aristi and Kefalovryso in rooting ability in the case of combine effect of multiplication and rooting media. 1 (MS5 + R1), 2 (MS5 + R2), 3 (MS5 + R3), 4 (MS9 + R1), 5 (MS9 + R2), 6 (MS9 + R3), Table S1: Substrate (medium) —Population interaction in terms of the number of newly formed shoots, Table S2. Substrate (medium) — population interaction in terms of shoot length, Table S3: Substrate — Variety Interaction in terms of rooting ability, Table S4: Interaction of Growth regulators and Population (Aristi and Kefalovryso) in the case of combine effect of multiplication and rooting medium.

Author Contributions: Conceptualization, S.K., S.H., I.N.-O. and A.K.K.; investigation, C.N., P.T., I.M., V.A., E.P., E.G. and I.N.-O.; plant material resources, S.K., E.G. and A.K.K.; methodology, C.N., P.T., S.K., S.H., I.M., E.P. and I.N.-O.; visualization, C.N., I.M., V.A. and E.P.; data curation, C.N., I.M., V.A. and E.P.; writing—original draft preparation, S.K., P.T., S.H. and I.N.-O.; writing—review and editing, S.K., P.T., I.N.-O., S.H. and A.K.K.; funding acquisition, S.K., A.K.K. and I.N.-O.; project administration, S.K. and A.K.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Programme Competitiveness, Entrepreneurship and Innovation, under the call "RESEARCH-CREATE-INNOVATE" (Project code: T1EDK-03919).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets in this paper are available from the corresponding authors on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Barnes, J.; Anderson, L.A.; Phillipson, J.D. *Herbal Medicines: A Guide for Healthcare Professionals*, 2nd ed.; Pharmaceutical Press: London, UK, 2002; 432p.
2. Akhondzadeh, S.; Noroozian, M.; Mohammadi, M.; Ohadinia, S.; Jamshidi, A.H.; Khani, M. *Salvia officinalis* extract in the treatment of patients with mild to moderate Alzheimer's disease: A double blind, randomized and placebo-controlled trial. *J. Clin. Pharm. Ther.* **2003**, *28*, 53–59. [CrossRef]
3. Gostin, I. Effects of different plant hormones on *Salvia officinalis* cultivated in vitro. *Int. J. Botany* **2008**, *4*, 430–436. [CrossRef]
4. Kintzios, S.; Nikolaou, A.; Skoula, M. Somatic embryogenesis and in vitro rosmarinic acid accumulation in *Salvia officinalis* and *S. fruticosa* leaf callus cultures. *Plant Cell Rep.* **1999**, *18*, 462–466. [CrossRef]
5. Azimova, S.S.; Glushenkova, A.I. *Lipids, Lipophilic Components and Essential Oils from Plant Sources*; Springer: London, UK, 2012; 1004p. [CrossRef]
6. Kontogianni, V.G.; Tomic, G.; Nikolic, I.; Nerantzaki, A.A.; Sayyad, N.; Stosic-Grujicic, S.; Tzakos, A.G. Phytochemical profile of *Rosmarinus officinalis* and *Salvia officinalis* extracts and correlation to their antioxidant and anti-proliferative activity. *Food Chem.* **2013**, *136*, 120–129. [CrossRef]
7. Modarres, M.; Esmaeilzadeh Bahabadi, S.; Taghavizadeh Yazdi, M.E. Enhanced production of phenolic acids in cell suspension culture of *Salvia leriifolia* Benth. using growth regulators and sucrose. *Cytotechnology* **2018**, *70*, 741–750. [CrossRef] [PubMed]
8. Brezoiu, A.M.; Prundeanu, M.; Berger, D.; Deaconu, M.; Matei, C.; Oprea, O.; Vasile, E.; Negreanu-Pîrjol, T.; Muntean, D.; Danciu, C. Properties of *Salvia offcinalis* L. and *Thymus serpyllum* L. extracts free and embedded into mesopores of silica and titania Nanomaterials. *Nanomaterials* **2020**, *10*, 820. [CrossRef]
9. Schmiderer, C.; Novak, J. Salvia officinalis L. and *Salvia fruticosa* Mill.: Dalmatian and three-lobed sage. In *Medicinal, Aromatic and Stimulant Plants*; Novak, J., Blüthner, W.D., Eds.; Handbook of Plant Breeding; Springer: Cham, Switzerland, 2020; Volume 12, pp. 523–527. [CrossRef]
10. Laurentin, H. Data analysis for molecular characterization of plant genetic resources. *Genet. Resour. Crop Evol.* **2009**, *56*, 277–292. [CrossRef]
11. Kostas, S.; Hatzilazarou, S.; Pipinis, E.; Vasileiadis, A.; Magklaras, P.; Smyrnioudis, I.; Vasilakis, T.; Chazakis, M.; Anastasiadi, V.; Ziogou, F.-T.; et al. Propagation of *Pistacia lentiscus* var. Chia genotypes and determination of their ornamental traits combined with a genetic analysis using ISSR markers. *Agronomy* **2021**, *11*, 205. [CrossRef]

12. Kostas, S.; Kaplani, A.; Koulaouzidou, E.; Kotoula, A.A.; Gklavakis, E.; Tsoulpha, P.; Hatzilazarou, S.; Nianiou-Obeidat, I.; Kanellis, A.K.; Economou, A. Sustainable exploitation of greek *Rosmarinus officinalis* L. populations for ornamental use through propagation by shoot cuttings and in vitro cultures. *Sustainability* **2022**, *14*, 4059. [CrossRef]
13. Nicola, S.; Fontana, E.; Hoeberechts, J.; Saglietti, D. Rooting products and cutting timing on sage (*Salvia officinalis* L.) propagation. *Acta Hortic.* **2003**, *676*, 135–141. [CrossRef]
14. Kara, N.; Baydar, H.; Erbaş, S. Effects of different cuttings periods and IBA concentrations on rooting ability of some medicinal plant. *Derim* **2011**, *28*, 71–81.
15. Paradikovic, N.; Zelkovic, S.; Tkalec, M.; Vinkovic, T.; Devic, I.; Maric, M. Influence of rooting powder on propagation of sage (*Salvia officinalis* L.) and rosemary (*Rosmarinus officinialis* L.) with green cuttings. *Poljoprivreda* **2013**, *19*, 10–15.
16. Loconsole, D.; Cristiano, G.; De Lucia, B. Image analysis of adventitious root quality in wild sage and glossy abelia cuttings after application of different indole-3-butyric acid concentrations. *Plants* **2022**, *11*, 290. [CrossRef]
17. Petrova, M.; Nikolova, M.; Dimitrova, L.; Zayova, E. Micropropagation and evaluation of flavonoid content and antioxidant activity of *Salvia officinalis* L. *Genet. Plant Physiol.* **2015**, *5*, 48–60. Available online: http://www.ifrg-bg.com (accessed on 19 June 2023).
18. Kriebel, R.; Drew, B.; González-Gallegos, J.; Celep, F.; Heeg, L.; Malik, M.; Sytsma, K. Pollinator shifts, contingent evolution, and evolutionary constraint drive floral disparity in *Salvia* (Lamiaceae): Evidence from morphometrics and phylogenetic comparative methods. *Evolution* **2020**, *74*, 1335–1355. [CrossRef] [PubMed]
19. Gokdogan, E.Y.; Burun, B. The studies on seed germination and in vitro cultures of *Salvia* L. species from turkish Flora. *Nat. Prod. Biotechnol.* **2022**, *2*, 60–73.
20. Grzegorczyk, I.; Bilichowski, I.; Mikiciuk-Olasik, E.; Wysokińska, H. In vitro cultures of *Salvia officinalis* L. as a source of antioxidant compounds. *Acta Soc. Bot. Pol. Pol. Tow. Bot.* **2005**, *74*, 17–21. [CrossRef]
21. Grzegorczyk-Karolak, I.; Matkowski, A.; Wysokińska, H. Antioxidant activity of extracts from in vitro cultures of *Salvia officinalis* L. *Food Chem.* **2007**, *104*, 536–541. [CrossRef]
22. Santos-Gomes, P.C.; Seabra, R.M.; Andrade, P.B.; Fernandes-Ferreira, M. Phenolic antioxidant compounds produced by in vitro shoots of sage (*Salvia officinalis* L.). *Plant Sci.* **2002**, *162*, 981–987. [CrossRef]
23. Santos-Gomes, P.C.; Fernandes-Ferreira, M. Essential oils produced by in vitro shoots of sage (*Salvia officinalis* L.). *J. Agric. Food Chem.* **2003**, *51*, 2260–2266. [CrossRef]
24. Wielgus, K.; Luwanska, A.; Szalata, M.; Mielcarek, S.; Gryszczynska, A.; Lipinski, D.; Slomski, R. Phytochemical estimation of Sage (*Salvia officinalis* L.) cultivated in vitro-flavonoids and phenolic acids. *Acta Fytotech. Zootech* **2011**, *14*, 8–11.
25. Bolta, I.; Evi, D.B.; Bohanec, B.; Andren, S. A preliminary investigation of ursolic acid in cell suspension culture of *Salvia officinalis*. *Plant Cell Tissue Organ Cult.* **2000**, *62*, 57–63. [CrossRef]
26. Mehalaine, S.; Chenchouni, H. New insights for the production of medicinal plant materials: Ex vitro and in vitro propagation of valuable Lamiaceae species from northern Africa. *Curr. Plant Biol.* **2021**, *27*, 100216. [CrossRef]
27. Zewdinesh, D.Z.; Beemnet, M.K. Effect of cutting size and position on propagation ability of Sage (*Salvia officinalis* L.). *Int. J. Adv. Biol. Biomed. Res.* **2016**, *4*, 68–76.
28. Murashige, T.; Skoog, F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant.* **1962**, *15*, 473–497. [CrossRef]
29. Esmaeili, G.; Fatemi, H.; Baghani avval, M.; Azizi, M.; Arouiee, H.; Vaezi, J.; Fujii, Y. Diversity of chemical composition and morphological traits of eight iranian wild *Salvia* species during the first step of domestication. *Agronomy* **2022**, *12*, 2455. [CrossRef]
30. Herraiz-Peñalver, D.; Asensio, S.; Manzanera, M.C.; Herrero, B.; Martin, H.; Santiago, Y.; Zalacaín, A.; Berruga, M.I.; Sánchez-Vioque, R. Evaluation of the morphological variability in Iberian *Salvia lavandulifolia* Vahl accessions. *Genet. Resour. Crop Evol.* **2017**, *64*, 1797–1806. [CrossRef]
31. Mossi, A.J.; Cansian, R.L.; Paroul, N.; Toniazzo, G.; Oliveira, J.V.; Pierozan, M.K.; Pauletti, G.; Rota, L.; Santos, A.C.A.; Serafini, L.A. Morphological characterisation and agronomical parameters of different species of *Salvia* sp. (Lamiaceae). *Braz. J. Biol.* **2011**, *71*, 121–129. [CrossRef]
32. Leontaritou, P.; Lamari, F.N.; Papasotiropoulos, V.; Iatrou, G. Exploration of genetic, morphological and essential oil variation reveals tools for the authentication and breeding of *Salvia pomifera* subsp. *calycina (Sm.) Hayek. Phytochemistry* **2021**, *191*, 112900. [CrossRef]
33. Leontaritou, P.; Lamari, F.N.; Papasotiropoulos, V.; Iatrou, G. Morphological, genetic and essential oil variation of greek sage (*Salvia fruticosa* Mill.) populations from Greece. *Ind. Crops Prod.* **2020**, *150*, 112346. [CrossRef]
34. Zigene, Z.D.; Asfaw, B.T.; Bitima, T.D. Phenotypic diversity of rosemary (*Salvia rosmarinus* Schleid.) accessions for qualitative characters. *Heliyon* **2022**, *8*, e11895. [CrossRef] [PubMed]
35. Araghi, A.M.; Nemati, H.; Azizi, M.; Moshtaghi, N.; Shoor, M.; Hadian, J. Assessment of phytochemical and agro-morphological variability among different wild accessions of *Mentha longifolia* L. cultivated in field condition. *Ind. Crops Prod.* **2019**, *140*, 111698. [CrossRef]
36. Devi, A.; Iqbal, T.; Wani, I.A.; Sharma, G.; Verma, S.; Noureldeen, A.; Darwish, H. Assessment of variability among morphological and molecular characters in wild populations of mint [*Mentha longifolia* (L.) L.] germplasm. *Saudi J. Biol. Sci.* **2022**, *29*, 3528–3538. [CrossRef]

37. Ayanoglu, F.; Özkan, C. Change in tissue mineral elemental concentration during root initiation and development of *Salvia officinalis* L. cuttings and effects. *Turk. J. Agric. For.* **2000**, *24*, 677–682.
38. Davies, F.; Geneve, R.; Wilson, S.; Hartmann, H.; Kester, D. *Hartmann and Kester's Plant Propagation: Principles and Practices*, 9th ed.; Pearson Prentice Hall: Upper Saddle River, NJ, USA, 2017; 915p.
39. Kacar, O.; Azkan, N.; Çöplü, N. 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.* **2009**, *7*, 349–352.
40. Martini, A.N.; Bertsouklis, K.F.; Vlachou, G.; Dariotis, E.; Papafotiou, M. Comparative evaluation of rooting cuttings of five Mediterranean sage species (*Salvia* sp.) native to Greece. *Acta Hortic.* **2020**, *1298*, 587–592. [CrossRef]
41. Vârban, D.I.; Vârban, R.; Duda, M.M.; Muntean, S.; Moldovan, C. The influence of substrates on rooting of *Salvia officinalis* L. cuttings. *Hop Med. Plants* **2014**, *24*, 52–56.
42. Lemraski, M.G.; Eftekhari, M.; Faraji, M.; Zarrini, S.S. Study of callus induction in common sage (*Salvia officinalis* L.). *Int. J. Agric. Crop Sci. (IJACS)* **2014**, *7*, 386–389.
43. Ioja-Boldura, O.M.; Radu, F.; Popescu, S.; Borozan, A. Regeneration, micropropagation, callus cultures and somatic embryogenesis of common sage (*Salvia officinalis* L.). *Bull. Univ. Agric. Sci. Vet. Med. Cluj-Napoca. Hortic.* **2010**, *67*, 308–313.
44. Villegas-Sánchez, E.; Macías-Alonso, M.; Osegueda-Robles, S.; Herrera-Isidrón, L.; Nuñez-Palenius, H.; González-Marrero, J. In vitro culture of *Rosmarinus officinalis* L. in a temporary immersion system: Influence of two phytohormones on plant growth and carnosol production. *Pharmaceuticals* **2021**, *14*, 747. [CrossRef]
45. Tsoulpha, P.; Alexandri, S.; Tsaktsira, M. Critical factors affecting an efficient micropropagation protocol for *Pyrus spinosa* Forskk. *J. Appl. Hortic.* **2018**, *20*, 190–195. [CrossRef]
46. Tawfik, A.A.; Mohamed, M.F. Regeneration of salvia (*Salvia officinalis* L.) via induction of meristematic callus. *In Vitro Cell. Dev. Biol. Plant* **2007**, *43*, 21–27. [CrossRef]
47. Ngomuo, M.; Mneney, E.; Ndakidemi, P.A. The in vitro propagation techniques for producing banana using shoot tip cultures. *Am. J. Plant Sci.* **2014**, *5*, 1614–1622. [CrossRef]
48. Rout, G.R.; Samantaray, S.; Das, P. In vitro manipulation and propagation of medicinal plants. *Biotechnol. Adv.* **2000**, *18*, 91–120. [CrossRef]
49. Mohamed, M.A.H.; Aly, M.K.; Ahmed, E.T.; Abd El-latif, S.A.H. Effect of plant growth regulators on organogenesis of *Salvia officinalis* L. plants. *Minia J. Agric. Res. Dev.* **2019**, *3*, 401–414.
50. Cuenca, S.; Amo-Marco, J.B. In vitro propagation of two spanish endemic species of *salvia* through bud proliferation. *In Vitro Cell. Dev. Biol. Plant* **2000**, *36*, 225–229. [CrossRef]
51. Arikat, N.A.; Jawad, F.M.; Karam, N.S.; Shibli, R.A. Micropropagation and accumulation of essential oils in wild sage (*Salvia fruticosa* Mill.). *Sci. Hortic.* **2004**, *100*, 193–202. [CrossRef]
52. Mascarello, C.; Mantovani, E.; Ruffoni, B. In vitro culture of several ornamental and medicinal *Salvia* species. *Acta Hortic.* **2006**, *723*, 375–380. [CrossRef]
53. van Staden, A.B.; Lall, N. Medicinal plants as alternative treatments for progressive macular hypomelanosis. In *Medicinal Plants for Holistic Health and Well-Being*, 1st ed.; Lall, N., Ed.; Elsevier Academic Press: London, UK, 2018; pp. 145–182.
54. Zuzarte, M.R.; Dinis, A.M.; Cavaleiro, C.; Salgueiro, L.R.; Canhoto, J.M. Trichomes, essential oils and in vitro propagation of *Lavandula pedunculata* (Lamiaceae). *Ind. Crops Prod.* **2010**, *32*, 580–587. [CrossRef]
55. Ghanbar, T.; Hosseini, B.; Jabbarzadeh, Z.; Farokhzad, A.; Sharafi, A. High-frequency in vitro direct shoots regeneration from axillary nodal and shoot tip explants of clary sage (*Salvia sclarea* L.). *Bulg. J. Agric. Sci.* **2016**, *22*, 73–78.
56. Jafari, S.; Daneshvar, M.H.; Salmi, M.S.; Abdi, L.J. Indirect organogenesis and plant regeneration in common sage (*Salvia officinalis* L.): An important medicinal plant of Iran. *Mod Appl. Sci.* **2017**, *11*, 22–29. [CrossRef]