Article

First Steps towards Pre-Breeding of *Sideritis scardica*: A Phenotypic, Agronomic, and Phytochemical Profiling Approach

Eirini Sarrou [1,†], Nektaria Tsivelika [1,†], Stefan Martens [2], Maria Irakli [1], Fotini Bletsaki [3], Sarra Broufa [3], Sampson Panajiotidis [3], Paschalina S. Chatzopoulou [1] and Eleni M. Abraham [3,*]

1 Hellenic Agricultural Organization—Dimitra (ELGO–Dimitra),
   Institute of Plant Breeding and Genetic Resources, 57001 Thessaloniki, Greece; esarroy@gmail.com (E.S.);
   riatsivel@gmail.com (N.T.); irakli@cerealinstitute.gr (M.I.); chatzopoulou@ipgrb.gr (P.S.C.)
2 Fondazione Edmund Mach, Department of Food Quality and Nutrition, 38010 Trento, Italy;
   stefan.martens@fmach.it
3 Faculty of Forestry and Natural Environment, Aristotle University of Thessaloniki,
   54124 Thessaloniki, Greece; fotinoulample@yahoo.gr (F.B.); mproufasarah@gmail.com (S.B.);
   pansamp@for.auth.gr (S.P.)
* Correspondence: eabraham@for.auth.gr
† These authors contributed equally to this work.

Abstract: *Sideritis scardica* (*S. scardica*) Griseb., also known as mountain tea, is an important medicinal and aromatic plant species. Due to the high cross-pollination ability of the species, diverse genotypes and phenotypes occur naturally. Considering that superior uniform genotypes are necessary for highly qualitative and sustainable production, this study aimed to conduct a pre-breeding evaluation of three clones (SID1, SID2, and SID3) originating from a selected *S. scardica* population growing in Greece. According to a phenotypic and agronomic evaluation, SID2 seemed to be superior among the three clones, expressing a good profile with desirable traits (i.e., desired inflorescence length and leaf surface, high length of stems, and high fresh and dry plant biomass). Furthermore, SID3 presented some remarkable measurements regarding morphological (upright growth habit) and agronomic (high number of stems and plant dry weight, desired plant surface) traits. The phytochemical profile of the three clones was assessed with regard to their volatile and polyphenolic compounds. Forty-four constituents were identified in *S. scardica* essential oil, including hydrocarbon monoterpenes, sesquiterpenoids, oxygenated monoterpenes, and other groups (monoterpene ketones, saturated fatty alcohols, benzoic esters). Liquid chromatographic analysis revealed SID2 as the clone most abundant in the major polyphenolic metabolites: verbascoside (2234.3 mg 100 g^{-1}), isoscutellarein-7-*O* [6″-*O*-acetyl]-allosyl(1 → 2) glucoside (1456.5 mg 100 g^{-1}), and 4-methyl hypolaetin-7-*O* [6″-*O*-acetyl]-allosyl(1 → 2) glucoside (861.8 mg 100 g^{-1}). The results indicate the ability to combine morphological, agronomic, and phytochemical traits, in order to identify superior *S. scardica* genotypes for further evaluation and utilization in breeding programs, aiming to create cultivars or varieties for farming purposes with desired performance and high quantitative and qualitative yields.

Keywords: mountain tea; morphological descriptors; biomass production; polyphenols; volatiles; non-metric multidimensional scaling

1. Introduction

Sideritis scardica Griseb. (Lamiaceae), also known as Mountain Tea, thrives in Balkan countries and is an important medicinal and aromatic plant (MAP) species. It is a perennial herbaceous species with branched and/or unbranched stems up to 40 cm high. The opposite leaves are covered by gray hairs, and the flowers form dense spikes with middle bracts of 12–20 mm in length. The cross-pollinated flowers develop a lemon-yellow glandular corolla, subtended by a tubular-campanulate calyx [1]. *S. scardica* is native in open, dry,

stony areas of subalpine and alpine vegetation belts in Greece, Albania, North Macedonia, Serbia, Bulgaria, and Turkey [2–4].

The aerial parts of *S. scardica* plants are utilized for the preparation of herbal tea, traditionally used for the symptoms of the common cold, cough, bronchitis, gastrointestinal disorders, etc. In addition to *S. scardica,* other species such as *S. raeseri*, *S. clandestina*, and *S. syriaca* are native to Greece and have also been recognized as traditional herbal medicines, indicating relief from the common cold and mild gastrointestinal disorders (EMA/HMPC/39455/2015, Committee on Herbal Medicinal Products (HMPC)). Many studies have documented biological activities such as anti-inflammatory, analgesic, antioxidant, and antimicrobial properties, as well as gastrointestinal protection properties, strongly supporting the abovementioned use of several *Sideritis* species [5–7]. Recently, the potential activity of *S. scardica* extracts in neurodegenerative diseases has been investigated more extensively [8,9]. The reported biological properties of *Sideritis* spp. and the extended phytochemical analysis of the genus initiated in the last decade, focusing mostly on the groups of terpenes (di- and triterpenes) and phenylpropanoids [5,6,10–14].

The essential oil of *S. scardica* is generally characterized by high contents of monoterpenes such as α- and β-pinene, sesquiterpenes (predominantly β-caryophyllene and α-cadinol), and lower amounts of oxygenated monoterpenes [15–17]. The non-polar extracts of *S. scardica* are rich in *ent*-kaurane diterpenes such as siderol, isolinearol, sideridiol, sideroxol, epoxysiderol, and 18-acetoxy-leucanthol [5,18], while triterpenes (ursolic and oleanolic acids) and phenylpropanoids (chlorogenic acid, verbascoside) have also been reported [19]. Furthermore, the qualitative polyphenolic fingerprint of alcoholic extracts has been well described, with acetylated and non-acetylated flavonoid-7-*O*-glycosides, various flavones (apigenin and luteolin derivatives), phenylethanoid glycosides, and hydroxycinnamic acids being the main components. The presence of two types of 7-*O*-glycosides of very rare 8-OH flavones—hypolaetin, isoscutellarein, and their methoxy derivatives—has been reported as representing the most species-characteristic compounds from the chemotaxonomic point of view [6,20,21].

The majority of the scientific reports on *S. scardica*'s pharmacological activities, which are attributed to the contents of phytochemicals, have resulted in a strong demand for high-quality and standardized raw plant materials. Due to the high cross-pollination ability between the different *Sideritis* species, diverse phenotypes are occurring as a result of cross-pollination. Natural populations of *S. scardica* in Greece and other Balkan countries are under threat due to the combined pressure imposed by reckless overharvesting of native plants, climate change, and variable (high/low) grazing intensity. Therefore, domestication of superior genotypes is necessary for highly qualitative and sustainable production that will provide a supply capable of meeting the increasing demand for raw material of the species. Furthermore, *S. scardica* is a drought-resistant species and could be an alternative crop for marginal environments, especially under the scenarios of future climatic fluctuations. Taking all of the above into consideration, the present study aimed to conduct a one-year pre-breeding evaluation of three clones developed after mass selection from a superior *S. scardica* population (expressing elongated inflorescences) originating from Northern Greece, with respect to their phenotypic and phytochemical characteristics.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Seeds from a native population of *S. scardica* growing in a mountainous area of Northern Greece (Menoikio Dramas; 41°11′44″ N, 23°46′08″ E) were collected in 2012 and germinated at the Institute of Plant Breeding and Genetic Resources (IPB&GR, Thessaloniki, Greece) in order to evaluate the putative domestication potential of the obtained seedlings. The species was authenticated by Dr. Theodoros Koutsos as *S. scardica* (Accession number MAPD 0003/GRC017). Fifty-five seedlings were transplanted in 2013 in the experimental field of IPB&GR (40°34′35″ N 22°57′19″ E) and were equally drip-irrigated and hand-weeded. The soil properties of the experimental field were as follows: soil type: red loam,

pH 7.7, clay: 39.0%, organic matter: 1.4%, P_2O_5: 45 ppm, K_2O: 520 ppm. During the second year of growth development (end of May 2015), three individual plants expressing superior agronomic and morphometric traits, such as erected plant habit, high density of branching, and long length of inflorescences, were selected in order to develop three clones through propagation of stem cuttings.

For the propagation, apical stem cuttings were collected from each individual plant during early autumn and, after treating their base with 1000 mg L^{-1} indol-3-butyric acid potassium salt, they were transplanted to plastic discs containing turf–perlite (3:1) for rooting. The discs with the stem cuttings were placed under an intermittent mist propagation bench in a greenhouse with an average temperature of 20 °C (± 2 °C) until desirable rooting was observed. Thirty-six plants per clone were transplanted in the experimental field (March 2016), with inter-plant and inter-row distances of 1.2 m and 2 m, respectively, in order to establish a completely randomized block design experiment (6 plants per block × 6 blocks per clone = total 108 plants).

Considering that *S. scardica herba* (the aerial parts of the plant) is utilized as a herbal preparation (EMA/HMPC/39453/201), and in order to assess possible phenotypic and chemotypic variation between the clones, principal morphometric, agronomic, and qualitative traits were evaluated for one year in the second year of the plants' development (May 2018), as follows:

(1) Morphological traits, focusing mainly on leaf and inflorescence size in order to develop a draft descriptor list for further breeding studies.

(2) Agronomic traits, referring to the plant's surface, number and length of stems, fresh and dry biomass production, etc.

(3) Qualitative traits: volatile and polyphenolic compounds.

2.2. Phenotypic and Agronomic Measurements

All morphometric and agronomic measurements were conducted at the stage of full blooming on an individual plant basis for the three clones. In order to ensure representative sampling, each plant was divided into four quadrants, and the evaluation was conducted with five stems per quadrant (20 stems per plant in total). The stems were hydrated for 24 h in water, and the evaluation of leaf and inflorescence morphological characteristics was conducted through Image Pro Plus Software (Media Cybernetics). In addition, stems were freeze-dried and milled to a fine powder for polyphenolic analysis.

During harvest period, all of the aerial parts were collected by cutting the stems five cm above the ground. The fresh weight of biomass and the dry weight after air-drying at ambient temperature under shade were recorded. Part of the dried plant material was used for the isolation of essential oils and the profiling of volatile compounds.

2.3. Essential Oil Isolation and Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

Dried aerial parts (consisting of inflorescences, leaves, and stalks) from six plants per block were pooled for each clone and subjected to hydrodistillation, using a Clevenger-type apparatus according to the European Pharmacopoeia. Fifty grams of dried plant material was distilled for 3 h. The obtained essential oil was diluted in 1 mL of pentane and dried over anhydrous sodium sulfate. Three hydrodistillations per block were conducted for each clone (3 clones × 6 blocks × 3 distillations = 54 samples in total).

The essential oils were directly injected and analyzed by GC/MS on a fused silica DB-5 column, using a gas chromatograph 17A Ver. 3 interfaced with a Shimadzu QP-5050A mass spectrometer (SHIMADZU EUROPA GmbH, Duisburg, Germany), supported by the GC/MS Solution Ver. 1.21 software, using a modified version of a method described previously [22]. The GC/MS analysis conditions were as follows: injection temperature: 260 °C, interface heating: 250 °C, ion source heating: 200 °C, EI mode: 70 eV, scan range: 41–450 amu, scan time: 0.50 s. The oven temperature program was as follows: 55°–95 °C (1.5 °C/min), 95°–120 °C (5 °C/min), 120–220 °C (3 °C/min), and 220–240 °C for 10 min. The carrier gas was He, at 54.8 kPa, and the split ratio was 1:20.

The relative content of each compound was calculated as percentage of the total chromatographic area, and the results were expressed as means of three replicates. The identification of the compounds was based on the comparison of their retention indices (RI) relative to *n*-alkanes (C7–C22) with corresponding literature data, and by matching their spectra with those of MS libraries (NIST 98).

2.4. Polyphenols Extraction

The extraction was accomplished by mixing 100 mg of freeze-dried tissues with 4 mL of 80% aqueous methanol in 15 mL Falcon tubes. The samples were sonicated for 15 min, mixed by an orbital shaker for 3 h at room temperature, and the extraction proceeded overnight at 4 °C in the dark. The resulting extracts were filtered through a MILLEX 13–0.22 µm PTFE membrane into glass vials and analyzed as described below.

2.5. Analysis of Phenolic Compounds and Flavonoid Aglycones

The phenolic compounds of the extracts were determined through HPLC analyses using an Agilent 1200 system (Agilent Technology, Urdorf, Switzerland), applying the chromatographic conditions described by Skendi et al. [23]. Chromatographic separation was carried out on a 250 mm × 4.6 mm Nucleosil 100 C_{18} 5 µm column at 30 °C. The mobile phase consisted of three solvents: 1% acetic acid in water (A), acetonitrile (B), and methanol (C). A linear gradient starting with 5% B and 5% C was installed to reach 4% B and 16% C at 10 min, 5% B and 20% C at 25 min, 5% B and 20% C at 30 min, 5% B and 30% C at 31 min, and finally 0% B and 60% C at 37 min. The flow rate was 1.3 mL min^{-1}, and the injection volume was 20 µL. Spectral data from all peaks were accumulated in the 240–400 nm range, and chromatograms were recorded at 260 nm for benzoic acid derivatives and kaempferol, while cinnamic acid derivatives and apigenin were analyzed at 320 and 330 nm, respectively. Quantification was performed using the corresponding calibration curves of phenolic compounds' external standards in the range between 0.5 and 20 ppm, and the results were expressed as mg 100 g^{-1} of dry weight of herbal mass. Eighteen samples per clone (random individuals from all six blocks) were analyzed, and the results were expressed as means.

2.6. Analysis of Flavones

The analysis of flavones was performed using a modified version of the method described previously by Rafique et al. [24]. Samples were directly injected after extraction. Ultra-high-performance liquid chromatography (UHPLC) was performed on a 1290 Infinity Binary UPLC (Agilent) equipped with a photodiode array (PDA). Separation of the flavones was achieved on a Macherey-Nagel Nucleodur C18ec column (4.6 µm, 250 mm × 4 mm), which was kept at 25 °C with solvent A (1% phosphoric acid in water) and solvent B (1% phosphoric acid in acetonitrile). The analysis and relative quantification of the flavones was performed under the following chromatographic conditions: 100% A to 50% A in 25 min, plateau of 3 min, up to 100% A in 7 min, and final plateau of 5 min, with a flow rate of 1 mL min^{-1}, and monitored at 222, 280, and 350 nm. In order to quantify the identified peaks, calibration curves were developed using external standards of the major compounds isoscutellarein-7-*O* [6''-*O*-acetyl]-allosyl(1 → 2) glucoside (IAAG) and 4-methyl hypolaetin-7-*O* [6''-*O*-acetyl]-allosyl(1 → 2) glucoside (mHAAG) (purchased from TransMIT PlantMetaChem, Giessen, Germany) at concentrations ranging between 30 and 350 ppm. A relative quantification was conducted for the remaining peaks assigned to isoscutellarein and hypolaetin derivatives due to their UV spectra, and their concentrations were expressed as mg 100 g^{-1} dry weight (DW).

2.7. Statistical Analysis

The statistical analysis of all data was performed using the SPSS V 18.0 package (SPSS Inc. Chicago, IL, USA). The data were analyzed with analysis of variance (ANOVA), and for the comparison of means, Tukey's test and standard error (S.E) were used at $p \leq 0.05$

to establish significant differences. In cases where the number of replicates was not equal, Welch's test was also applied in order to evaluate the significance of ANOVA.

Differences in the essential oil chemical profiles of all clones and blocks were detected using non-metric multidimensional scaling (NMS) and relative Euclidean distance (chord distance) as the original metrics of distance among them. NMS is widely used in ecology, as it is not sensitive to normality criteria [25]. As the main matrix, we chose essential oil components with detected presence >2% in more than half of the whole block, and as the secondary matrix, we chose the total of the morphometric and agronomic traits. NMS facilitated ordination of the three *S. scardica* clones and their blocks in the essential oil component space. It preserved the structure of the original multidimensional space and allowed its representation in a space of fewer dimensions. NMS does not assume linearity between essential oil components and morphological traits, and it exposes relationships between the two matrices. NMS was performed with 200 random starts, and ties were not penalized. A randomization procedure was included to test whether the solutions were stronger than those obtained by chance. The degree of preservation of structure in the original space (original variance) by the space of the NMS solution (fewer dimensions) was assessed with R^2 values. The relationships of each axis with essential oil components and morphological traits were quantified with Pearson correlation coefficients. All analyses were carried out with PC-ORD 7.0 software [26].

3. Results

3.1. Main Agronomic and Phenotypic Characteristics of the Three Clones of S. scardica

The main agronomic characteristics of the selected clones of *S. scardica* cultivated under the same environmental conditions are presented in Table 1. The plant surface varied from 1 to 1.2 m^2 among the clones, indicating that SID3, followed by SID2, was superior. Generally, SID2 seemed to be the most highly rated clone, as it exhibited significantly higher stem length and plant fresh biomass compared to the other two clones, and at the same time presented some of the highest values for the other agronomic traits. No significant differences were noticed in the number of stems among the three clones of *S. scardica*. Moreover, it was observed that SID1 had higher variation in plant surface, number of stems, and plant dry biomass compared to the other clones, as measured by coefficients of variation. In contrast, SID3 consisted of less variable individual plants and, thus, represented greater homogeneity with respect to these three main agronomic traits.

Table 1. The main agronomic traits of the three *S. scardica* clones and their coefficients of variation (CVs).

Clone	Plant Surface (m^2)	Number of Stems	Stem Length (cm)	Plant Fresh Weight (g)	Plant Dry Weight (g)
SID1	1.0 ± 0.2 [b]	232.0 ± 89.0 [a]	45.0 ± 5.3 [b]	1789.6 ± 695.9 [b]	454.5 ± 218.7 [b]
SID2	1.1 ± 0.2 [ab]	277.0 ± 85.0 [a]	55.7 ± 7.3 [a]	2968.1 ± 1055.8 [a]	636.1 ± 270.7 [a]
SID3	1.2 ± 0.2 [a]	261.0 ± 77.0 [a]	44.7 ± 5.6 [b]	1760.1 ± 722.6 [b]	502.2 ± 160.8 [ab]
CV (%)					
SID1	20.5	38.4	11.8	38.9	48.1
SID2	18.2	30.9	13.1	35.6	42.6
SID3	16.0	29.9	12.5	41.1	30.0

Values refer to the means ± standard deviations of the replicates. The replicates per clone were n = 29–36 individual plants. Different letters within the columns indicate significant differences according to Tukey's test for $p \leq 0.05$.

In addition to the agronomic traits related to the yield of each clone, a descriptor list was developed, describing the individual plants' morphology, in order to characterize the clones and determine the extent to which each characteristic is influenced by genetic or environmental factors (Table 2). According to the results, there was uniformity in most of the traits based on visual assessment. All three *S. scardica* clones presented medium plant width and shoot density, along with a dense pubescence of the stems. Regarding

the leaf characteristics, they all had a simple leaf type, the broadest part of the leaf blade was at the middle, the shape of the base and apex was acute, the main color was gray-green, and the incision and undulation of the margin were absent (or very weak) and medium, respectively. Furthermore, as with the stems, the leaf blades were also dense and pubescent in all three clones. Some major inflorescence characteristics were also shown to be stable regardless of the *S. scardica* clone. Specifically, all three clones showed absence of inflorescence internodes, and the main color of the outer side for both bracts and calyxes was light green. Moreover, the pubescence on the outer side of calyxes appeared dense for all three clones. The corolla tube and the upper and lower lip of the flowers of the inflorescences had a light yellow color for all of the clones, while the pubescence on the outer side of the upper lip was sparse. Finally, the width of the flower's lower lip was medium, the attitude relative to the corolla tube was moderately downwards, and there was no secondary color of the inner lower lip side for the majority of the plants of the three clones.

Table 2. Morphological characteristics of the three *S. scardica* clones.

	Morphological Characteristics	SID1	SID2	SID3
Plant				
1	Growth habit	Semi-upright *	Semi-upright	Upright
2	Height	Tall	Tall	Medium
3	Width	Medium	Medium	Medium
4	Density of shoots	Medium	Medium	Medium
5	Stem: pubescence	Dense	Dense	Dense
Leaf				
6	Type	Simple	Simple	Simple
7	Petiole: length (mm)	2.7 **	2.9	1.9
8	Leaf blade: length (cm)	6.5	6.7	6.3
9	Leaf blade: width (cm)	1.0	0.9	1.2
10	Leaf blade: length/width ratio	6.9	7.7	5.3
11	Leaf blade: position of broadest part	At middle	At middle	At middle
12	Leaf blade: shape of base	Acute	Acute	Acute
13	Leaf blade: shape of apex	Acute	Acute	Acute
14	Leaf blade: main color	Gray-green	Gray-green	Gray-green
15	Leaf blade: pubescence	Dense	Dense	Dense
16	Leaf blade: incision of margin	Absent or very weak	Absent or very weak	Absent or very weak
17	Leaf blade: undulation of margin	Medium	Medium	Medium
Inflorescence				
18	Length (cm)	20.3	23.3	18.6
19	Length of internode	Absent	Absent	Absent
20	Number of florets per node	6	6	6
21	Number of lateral branches	Absent or very few	Few	Medium
22	Attitude of tip	Erect	Semi-erect	Erect
23	Bract: length (cm) ***	2.1	2.1	2.2
24	Bract: main color of outer side	Light green	Light green	Light green
25	Calyx: length (cm)	0.7	0.7	0.7
26	Calyx: main color of outer side	Light green	Light green	Light green
27	Calyx: pubescence on outer side	Dense	Dense	Dense
28	Corolla: length (cm)	1.0	1.1	1.1
29	Corolla: height (cm)	0.4	0.4	0.4
30	Corolla tube: main color of outer side	Light yellow	Light yellow	Light yellow
31	Upper lip: main color of outer side	Light yellow	Light yellow	Light yellow
32	Upper lip: pubescence on outer side	Sparse	Sparse	Sparse
33	Lower lip: width	Medium	Medium	Medium
34	Lower lip: attitude relative to corolla tube	Moderately downwards	Moderately downwards	Moderately downwards
35	Lower lip: main color of inner side	Light yellow	Light yellow	Light yellow
36	Lower lip: secondary color of inner side	Absent	Absent	Absent

* Based on visual assessment of the majority of plants for each clone. ** Average of the individual measurements for each clone. *** Measurement in the middle part of the inflorescence.

Apart from the similar morphological traits for the three *S. scardica* clones, there were some differences regarding the plant height, with the two clones having a semi-upright growth habit and being tall (SID1, SID2), while SID3 had an upright growth habit and medium height. The inflorescences of the three clones also presented different tip attitudes, with SID1 and SID3 being erect, while SID2 was semi-erect (Supplementary Figure S1). The number of lateral inflorescence branches was absent or very few for SID1, few for SID2, and medium for SID3. Additionally, differences were observed in the time of blooming and the pattern of flowering among the clones: SID1 bloomed first, followed by SID2 (10 days after SID1), while SID3 flowered last (7 days after SID2) (Figure 1 and Table 2).
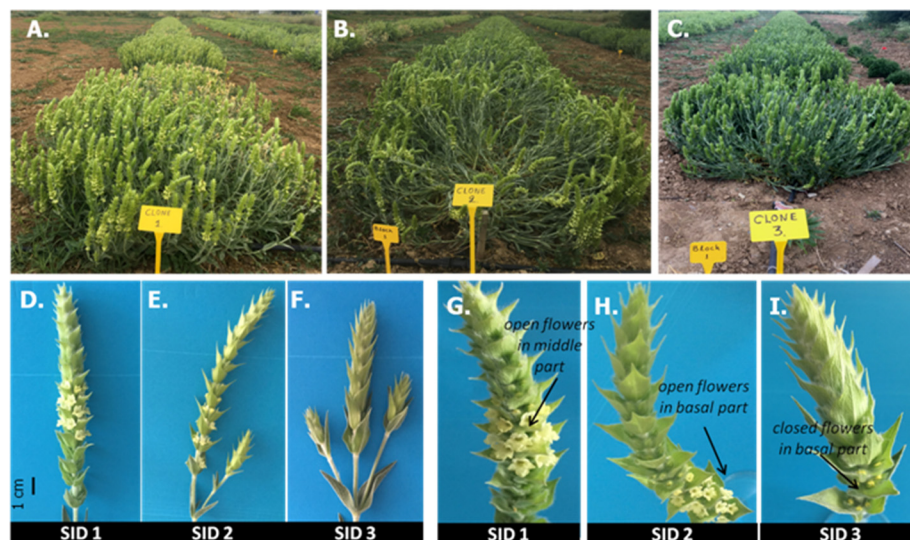

Figure 1. The experimental block design of the three *Sideritis scardica* clones cultivated in the IPB&RG farm (**A–C**). Representative inflorescences from the clones (SID1–SID3), with their descriptive differences in length and branching layout shown in the pictures (**D–F**), while zooming in (**G–I**) shows the different stages of blooming (photos were taken in early May).

With regard to measurable characteristics, the mean values of the number of florets per node, the length of bracts and calyxes, and the height and length of corollas were similar for the three clones. The leaf blade length/width ratio showed the highest value (7.7) for SID2 and the lowest (5.3) for SID3. The length of the leaf petioles and leaf blades also differed, with SID2 having the longest (2.9 mm and 6.7 cm, respectively) and SID3 the shortest (1.9 mm and 6.3 cm, respectively). The width of the leaf blades ranged between 0.9 and 1.2, with SID2 having the smallest and SID3 the highest. SID2 also presented the longest inflorescences (23.3 cm), while SID3 had the shortest (18.6 cm) (Table 2).

The plant organs that are utilized for herbal preparations and also contribute to the plant's productivity, i.e., inflorescences and leaves, were evaluated in detail in order to access putative variation among the three clones. The results presented in Table 3 show significant morphometric differences regarding the surface and the perimeter of the leaves, as well as similar mean values for the length and width of leaves and the length of inflorescences. Generally, clone SID2 presented more elongated inflorescences, while SID1 expressed an intermediate inflorescence length. In addition, SID2 exhibited a significantly larger leaf surface and perimeter in comparison to SID1 and SID3, while the latter two did not differ from each other. According to the coefficient of variation, SID2 gave relatively low percentages for most of the characteristics, indicating homogeneity between the plants and indirectly implying that the good morphological performance is mainly due to genetic and secondarily to environmental factors.

Table 3. The main morphometric traits of the leaves and inflorescences of the three *S. scardica* clones.

	Inflorescence		Leaves		
Clone	**Length (cm)**	**Surface (cm²)**	**Perimeter (cm)**	**Length (cm)**	**Width (cm)**
SID1	20.3 ± 1.7 [a]	4.6 ± 2.8 [b]	13.1 ± 4.9 [b]	6.5 ± 0.9 [a]	1.0 ± 0.1 [a]
SID2	23.3 ± 2.4 [a]	5.0 ± 2.1 [a]	14.1 ± 5.3 [a]	6.7 ± 1.2 [a]	0.9 ± 0.2 [a]
SID3	18.6 ± 4.6 [a]	4.6 ± 2.0 [b]	13.2 ± 4.8 [b]	6.3 ± 1.4 [a]	1.2 ± 0.2 [a]
			CV%		
SID1	8.4	60.9	37.4	14.3	13.6
SID2	10.2	42.0	37.6	18.5	24.0
SID3	24.8	43.5	36.4	22.4	20.0

Values refer to means ± standard deviations of replicates. The replicates per clone were n = 29–36 plants × 20 measurements per plant. Different letters within the columns indicate significant differences according to Tukey's test for $p \leq 0.05$.

3.2. Phytochemical Evaluation of the Three S. scardica Clones and NMS Ordination

The phytochemical evaluation of the three clones, enabling analysis of volatile and polyphenolic compounds, was carried out in order to identify putative differences between the clones. Concerning the volatile fingerprints of the three clones, forty-four compounds (accounting for 87.54–92.78% of the total essential oil) were identified in *S. scardica* essential oil, mostly including hydrocarbon monoterpenes, sesquiterpenoids, oxygenated monoterpenes, and other groups (monoterpene ketones, saturated fatty alcohols, benzoic esters) (Figure 2 and Table 4). The three clones exhibited similar qualitative profiles, although significant differences in the composition of essential oils were observed. SID1 represented higher accumulation of hydrocarbon monoterpenes compared to SID2 and SID3, which did not differ from each other (Figure 2). Conversely, SID2 expressed higher contents of sesquiterpenoids in comparison to SID1, but without differing significantly from SID3. Nevertheless, SID3 represented 3-fold higher contents of oxygenated monoterpenes compared to SID1 and SID2, while no significant variation was observed between the latter two. As regards the individual volatile components, 43 compounds were identified in the essential oil of SID1 (missing benzyl benzoate), 39 compounds were identified in SID2 (2.4 thujadiene, *cis* verbenol, *tr*-verbenol, *p*-mentha-1.5dien8ol, and benzyl benzoate were absent), and 42 were found in SID3 (without geranyl-*p*-cymene and *α*-bisabolene) (Table 4).
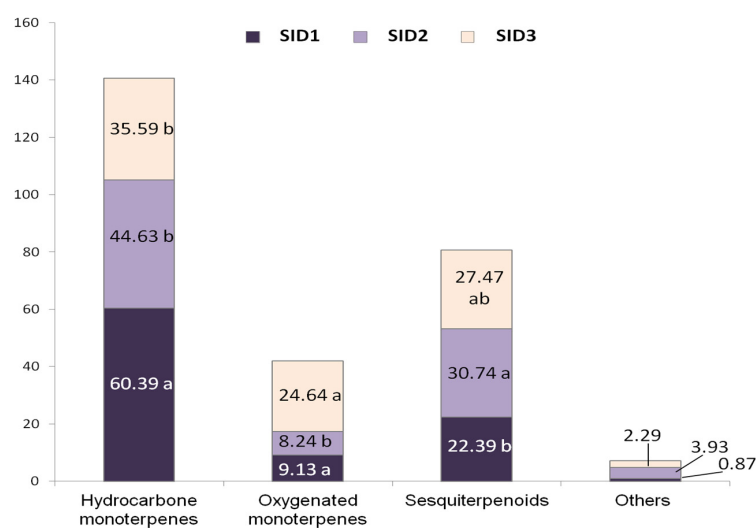

Figure 2. Differences in the % contents of groups of volatile compounds among the three *S. scardica* clones. The bar indicated as "Others" contains compounds belonging to monoterpenes, ketones, saturated fatty acids, and benzoic esters. All data represent the mean values. Values with the same letter within the bars with the same color are not statistically different according to Tukey's test for $p \leq 0.05$.

Table 4. Composition (%) of the essential oil from the three *S. scardica* clones.

	Compounds	SID1	SID2	SID3
Hydrocarbon monoterpenes		%		
1	*a*-Pinene	31.26 ± 0.27 [a]	7.94 ± 0.63 [c]	12.45 ± 1.62 [b]
2	2.4 Thujadiene	0.39	nd	0.75
3	Sabinene	1.14	2.25	0.72
4	*β*-Pinene	14.34 ± 0.29 [a]	16.43 ± 1.30 [a]	7.52 ± 0.64 [b]
5	1-Octen-3-ol	4.1 ± 0.14 [a]	5.54 ± 0.64 [a]	5.89 ± 0.58 [a]
6	*β*-Myrcene	0.94	0.84	0.64
7	*p*-Cymene	0.25	0.07	1.82
8	Limonene	2.47 ± 0.06 [b]	5.98 ± 0.58 [a]	2.69 ± 024 [b]
9	*β*-Phellandrene	0.74	0.94	0.75
10	*γ*-Terpinene	0.19	0.48	0.40
11	*α*-Terpinolene	0.14	0.26	0.23
12	*cis β*-Ocimene	3.62	3.34	1.31
13	*tr β*-Ocimene	0.71	0.50	0.19
Oxygenated monoterpenes				
14	1.8 Cineol	0.10	0.05	0.22
15	Linalool	0.78	1.53	1.07
16	*n*-Nonalal	0.28	0.50	0.35
17	*α*-Camphonelal	0.82	0.24	2.11
18	*tr* Pinocarveol	0.84	0.30	2.74
19	*cis* Verbenol	0.39	nd	1.13
20	*tr* Verbenol	0.91	nd	4.25
21	Pinocarvone	0.78	0.31	2.59
22	*p* Mentha-1.5dien8ol	0.38	nd	0.77
23	4 Terpineol	0.19	0.4	0.42
24	Myrtenol	1.37 ± 0.09 [b]	0.83 ± 0.10 [b]	4.36 ± 0.20 [a]
25	Thymol	0.21	0.53	0.42
26	Carvacrol	2.2 ± 0.2 [a]	3.61 ± 0.12 [a]	4.43 ± 1.0 [a]
Sesquiterpenes				
27	*δ*-Elemene	0.34	0.39	0.31
28	*α*-Copaene	0.08	0.32	0.13
29	*β*-Bourbonene	0.44	0.72	1.34
30	*β*-Cubebene + β-Elemene	0.28	0.51	0.77
31	*β*-Caryophyllene	9.59 ± 0.90 [a]	5.73 ± 0.45 [b]	7.35 ± 0.36 [ab]
32	*α*-Humulene	0.73	0.73	0.32
33	*D* Germacrene	3.65	1.58	2.53
34	*β*-Germacrene	2.35 ± 0.32 [b]	6.03 ± 0.46 [a]	3.31 ± 0.13 [ab]
35	*β*-bisabolene	0.52	0.42	0.28
36	*δ*-Cadinene	0.12 ± 0.05 [b]	6.24 ± 0.48 [a]	0.24 ± 0.01 [b]
37	Spathulenol	1.15 ± 0.08 [c]	3.07 ± 0.31 [b]	4.83 ± 0.36 [a]
38	Caryophyllene oxide	2.86 ± 0.07 [b]	1.56 ± 0.23 [b]	5.85 ± 0.53 [a]
39	Viridiflorol	0.08	0.26	0.23
40	*α*-Bisabolene	0.20	0.19	nd
Others				
41	Geranyl *p* cymene	0.58	1.25	nd
42	Geranyl acetone	0.11	0.94	0.49
43	Tetradecanol	0.18	1.74	0.73
44	Benzyl benzoate	nd	nd	1.06
Total identified essential oil %		92.78	87.54	89.99

All data represent the mean values (±standard error); significant differences are given for the compounds with values > 5% ± standard error; n = 3 biological replicates/distillations per clone. Values with the same letter within the rows are not statistically different according to Tukey's test for $p \leq 0.05$; nd, not detected.

The eleven essential components that met the criterion set for the NMS analysis (>2% in more than 50% of all blocks) are shown in Figure 3. Except for block 4 of SID2 (SID24, 54%), they constituted 62% to 83% of the total detected components in all blocks of the three clones. Clone SID1 (80–83%) represented higher concentrations, while the other two were lower and rather similar (SID2: 66–69%, SID3: 62–67%). The NMS randomization procedure provided a stable two-dimensional solution (final stress = 1.91, final instability = 0, p = 0.02). The two axes accounted for 98% (cumulative R^2 = 0.98) of the original variance, with Axis1 and Axis2 accounting for 60% and 38%, respectively. Except for block 4 of SID2 (SID24), all three clones form variably dense clusters that are clearly separated (Figure 3). Axis1 separates SID1 from clones SID2 and SID3 while Axis2 further separates the latter two. Axis1 is characterized by high negative $α$-pinene (r = −0.959) and positive B-germacrene (r = 0.841) association, while Axis2 is characterized by high negative $β$-pinene (r = −0.927), limonene (r = −0.836), and *cis-β*-ocimene (r = −0.837), high positive caryophyllene oxide (r = 0.892), and relatively high positive spathulenol (r = 0.692) associations (Supplementary Table S1). On the topology of the NMS graph, the Hilltop overlays represent areas of the graph with the highest 20% of the range of the chosen essential components, i.e., $α$- and $β$-pinene, caryophyllene oxide, limonene, and spathulenol (Figure 3). Axis1 is weakly associated with plant dry weight (r = 0.472) and moderately associated with plant fresh weight (r = 0.661) and leaf width (r = 0.533). The shoot length is moderately associated with both axes (Axis1 positively, Axis2 negatively). However, the inflorescence length is the morphological trait distinguished for its high negative association (r = −0.762) with Axis2 (Supplementary Table S1).
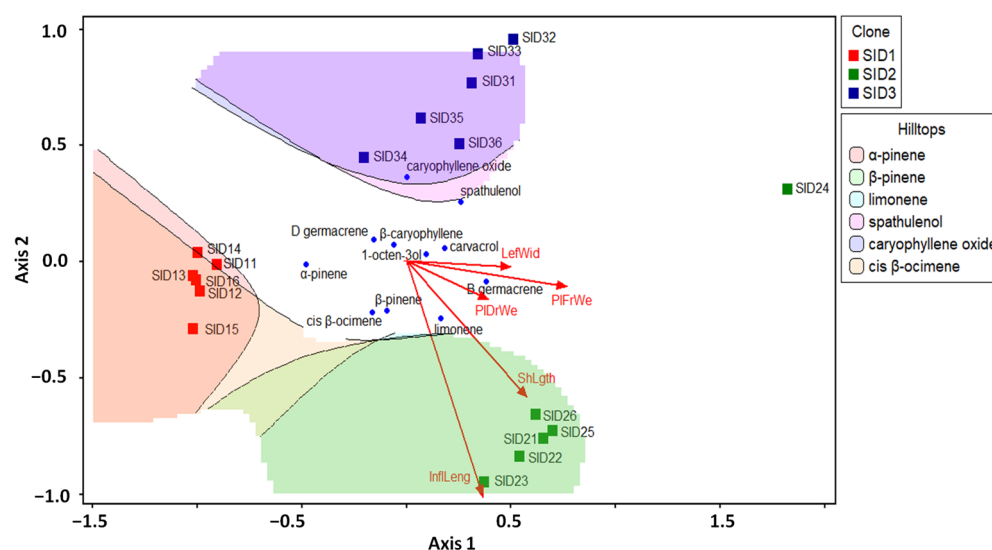

Figure 3. NMS of the three *S. scardica* clones based on major essential oil components, and correlation of its axes with five morphological traits (InflLength: inflorescence length, ShLgth: stem length, PlDWe: plant dry weight, PlFrWe: plant fresh weight, LefWid: leaf width). Hilltops represent areas in the diagram where specific compounds show the highest 20% of their range.

Concerning the polyphenolic analysis, seventeen compounds belonging to five main groups—hydroxybenzoic acids, hydroxycinnamic acids, flavonols, phenylethanoid glycosides, and flavones—were identified and quantified (Tables 4 and 5) through HPLC-DAD. Generally, all *Sideritis* extracts had a similar qualitative polyphenolic profile, with the most predominant constituents identified as chlorogenic acid and verbascoside, with concentrations varying in the ranges of 134.88–230.00 mg 100 g^{-1} and 1511.51–2234.32 mg 100 g^{-1} among the clones, respectively (Table 5). Comprehensive attention was given to a group of species-specific flavones, with isoscutellarein and hypolaetin derivatives as predominant ones (Table 6 and Figure 4). All *Sideritis* extracts were characterized by the presence of different derivatives of these two flavones. Eight peaks corresponding to isoscutellarein

(peaks 1, 3, 4, 6, and 7) and hypolaetin derivatives (peaks 2, 5, and 8) were identified according to their relative spectra, and according to previous reports (Figure 3). The most predominant peaks (six out of eight) were relatively quantified with the two available standard compounds IAAG (corresponding to peak 4) and mHAAG (corresponding to peak 5). Among the isoscutellarein derivatives, those corresponding to peaks 1 and 4 were the major ones in all of the examined extracts, with concentrations varying among the clones in the ranges of 371.9–634.5 and 913.3–1456.5 mg 100 g^{-1}, respectively. For hypolaetin derivatives, peaks 2 and 5 were the most dominant, with relative concentrations ranging from 339.8 to 385.8 and from 614.1 to 861.8 mg 100 g^{-1}, respectively (Table 6). Significant quantitative differences were recorded among the clones, with SID2 having the higher contents of the main flavones related to peaks 4 and 5. On the other hand, the three clones did not show significant differences concerning the contents of flavones related to peaks 2 and 7. In general, SID2 was identified as the superior clone concerning the total contents of isoscutellarein, hypolaetin derivatives, and their sum, while SID3 was characterized by significantly lower concentrations of the respective flavones.

Table 5. The phenolic acid, flavonol, flavone, and phenylethanoid glycoside compositions of the three *S. scardica* clones.

	Clone		
Compounds	**SID1**	**SID2**	**SID3**
Hydroxybenzoic acids	Concentration (mg 100 g^{-1})		
p-Hydroxybenzoic acid	7.41 ± 0.07 [a]	7.09 ± 0.10 [a]	7.24 ± 0.13 [a]
Protocatechuic acid	9.27 ± 0.28 [b]	11.41 ± 0.26 [a]	9.33 ± 0.17 [b]
Syringic acid	5.18 ± 0.13 [a]	5.42 ± 0.33 [a]	4.70 ± 0.22 [a]
Vanillic acid	11.13 ± 0.29 [a]	10.61 ± 0.30 [a]	8.36 ± 0.41 [b]
Hydroxycinnamic acids			
Chlorogenic acid	134.88 ± 4.69 [b]	229.03 ± 4.67 [a]	230.00 ± 12.97 [a]
Cryptochlorogenic acid	0.35 ± 0.08 [b]	0.56 ± 0.13 [a]	0.46 ± 0.11 [a]
Caffeic acid	7.91 ± 0.15 [b]	8.82 ± 0.23 [a]	7.96 ± 0.13 [b]
Flavonols			
Kaempferol	19.27 ± 0.58 [a]	9.61 ± 0.44 [c]	11.55 ± 0.64 [b]
Flavones			
Apigenin	6.55 ± 1.33 [a]	4.82 ± 1.87 [a]	11.29 ± 3.55 [a]
Phenylethanoid glycosides			
Verbascoside	1989.87 ± 89.93 [a]	2234.32 ± 71.91 [a]	1511.51 ± 130.64 [b]

All data represent the mean values ± standard error; $n_{(individuals)}$ = 18 biological replicates. Values with the same letter within the rows are not statistically different according to Tukey's test for $p \leq 0.05$.

Table 6. Composition of the species-characteristic flavones (isoscutellarein and hypolaetin derivatives) identified in the three *S. scardica* clones.

	Clone		
Flavones	**SID1**	**SID2**	**SID3**
Isoscutellarein derivatives	Concentration (mg 100 g^{-1})		
Peak 1	634.5 ± 51.3 [a]	485.9 ± 26.7 [b]	371.9 ± 20.1 [b]
Peak 3	60.5 ± 6.9	98.0 ± 13.2	32.7 ± 6.6
Peak 4 (IAAG)	1239.6 ± 78.2 [a]	1456.5 ± 61.4 [a]	913.31 ± 49.4 [b]
Peak 6	77.2 ± 7.5 [a]	31.2 ± 8.2 [b]	32.3 ± 9.7 [b]
Peak 7	45.8 ± 5.2 [a]	26.9 ± 10.5 [a]	38.1 ± 13.7 [a]
Hypolaetin derivatives			
Peak 2	364.4 ± 21.8 [a]	385.8 ± 23.6 [a]	339.8 ± 24.7 [a]
Peak 5 (mHAAG)	720.5 ± 33.8 [b]	861.8 ± 36.2 [a]	614.1 ± 36.0 [b]
Total isoscutellarein derivatives	2057.7 ± 58.7 [a]	2094.8 ± 77.3 [a]	1372.6 ± 78.2 [b]
Total hypolaetin derivatives	1084.9 ± 30.2 [b]	1247.6 ± 49.2 [a]	954.0 ± 51.8 [b]
Total identified flavones	3142.3 ± 85.5 [a]	3342.4 ± 124.8 [a]	2937.2 ± 126.3 [b]

All data represent the mean values ± standard error; $n_{(individuals)}$ = 18 biological replicates. Values with the same letter within the rows are not statistically different according to Tukey's test for $p \leq 0.05$. IAAG: isoscutellarein-7-*O* [6′′-*O*-acetyl]-allosyl(1 → 2) glucoside; mHAAG: 4-methyl hypolaetin-7-*O* [6′′-*O*-acetyl]-allosyl(1 → 2) glucoside.
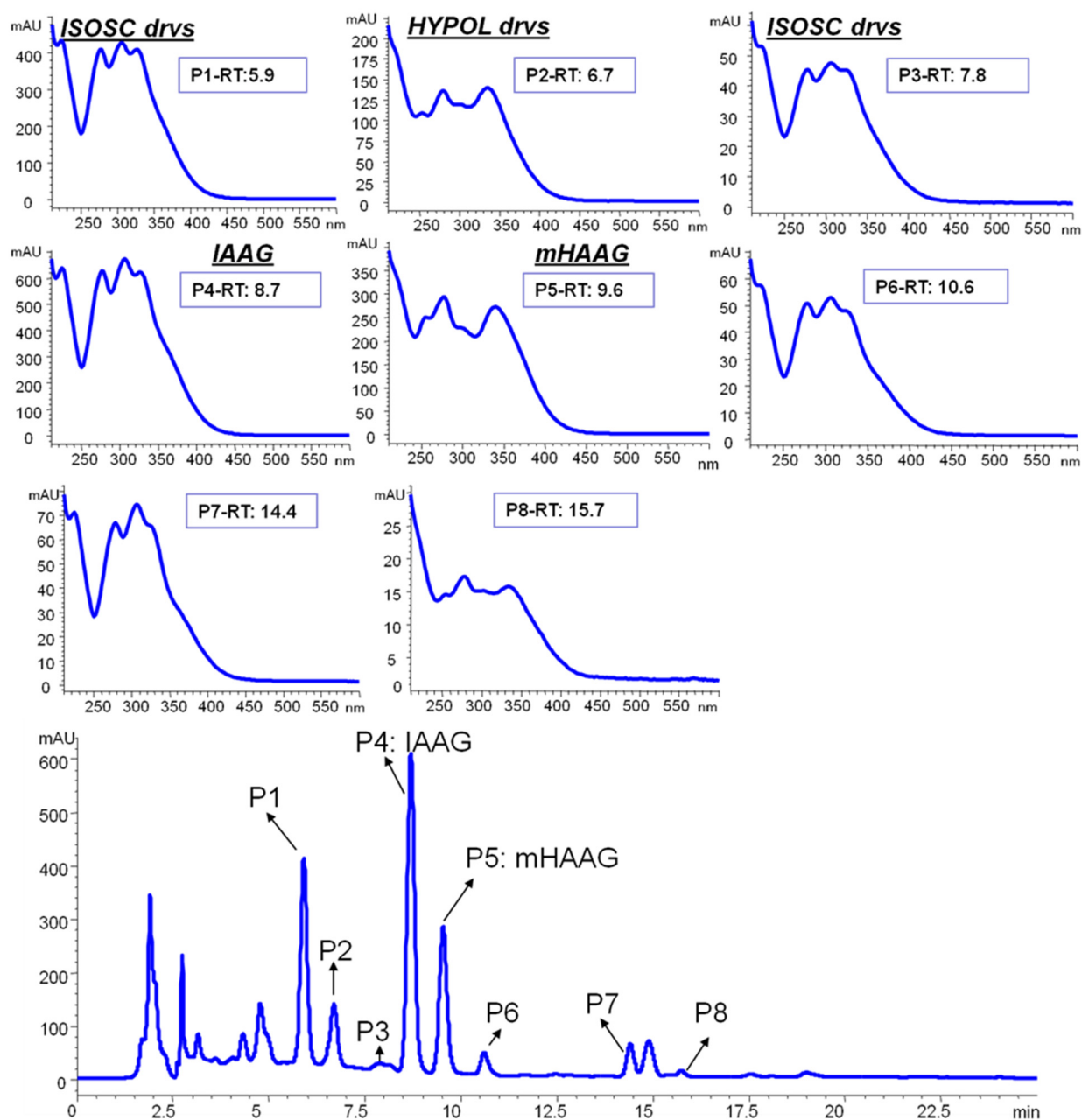
Figure 4. Descriptive UPLC chromatogram of *S. scardica herba* extracts with the characteristic flavones of the species, and the relative spectra of isoscutellarein derivatives (ISOSCU drvs) and hypolaetin derivatives (HYPOL drvs). IAAG: isoscutellarein-7-*O* [6″-*O*-acetyl]-allosyl(1 → 2) glucoside; mHAAG: 4-methyl hypolaetin-7-*O* [6″-*O*-acetyl]-allosyl(1 → 2) glucoside.

4. Discussion

Under a climate change environment, introducing *Sideritis* species into cultivation faces many critical challenges, including the selection of appropriate plant germplasm, susceptibility to fungal diseases, crop modernization through the development of harvest machinery, market fluctuations, etc. Today, natural *Sideritis* populations face the danger of extinction due to climate changes such as lack of precipitation and increased temperatures, but also anthropogenic impacts (overharvesting, overgrazing, etc.). On the other hand, the great phenotypic variability observed in wild-growing plants, but also in cultivated ones, is possibly associated with chemovariability, thus causing the quality of the herbal products to fluctuate. Moreover, the pharmaceutical industry prefers cultivated plant

material, as it makes it easier to achieve appropriate yield and uniformity, and to provide a constant quality and drug composition. Furthermore, through cultivated plant material, the possibility of plant misidentification and adulteration is almost excluded.

S. scardica is used for the preparation of a popular traditional tea/beverage that is considered to be a great source of "health-promoting" constituents. Beyond that, lately it has attracted the interest of many industries as a source of novel ingredients for food supplements with health benefits, as well as for cosmetics and pharmaceuticals. Despite the fact that the phytochemical patterns of *S. scardica* of different origins have been extensively described in the last two decades, reports on pre-breeding of the *Sideritis* species, and on the evaluation of morphological, agronomic, and phytochemical traits in cultivated plants, are scarce. To the best of our knowledge, this is the first study on pre-breeding evaluation of *S. scardica*, aiming to introduce superior genotypes into cultivation. In addition, *S. scardica* is considered to be a near-threatened species (IUCN red list); therefore, cultivation instead of wild harvesting could promote population sustainability and preservation (ex situ).

Similar to other wild MAPs, the successful domestication of *S. scardica* will enable the production of uniform and homogeneous plant material, while also contributing to the optimization of yields, improvement of crude plant extract quality, and the standardization of bioactive compound contents, as well as taking into consideration the market demands for homogeneous raw material [27,28].

In the present study, the main agronomic traits concerning the aerial parts and organs of *S. scardica*, commonly utilized by the industry, were evaluated. In contrast to morphological traits, most of the agronomic ones presented significant differences. This might be related to the progenitor of each clone. The vigor and the yield of each progenitor and the percentage of heritability for each agronomic trait can influence the performance and yield of the clones' progeny. In the absence of a draft descriptor list for *S. scardica*, or even for related species, this study evaluated the main morphological characteristics, revealing a uniformity between the three clones for most of them, which strengthens the possibility that these traits might be mainly influenced by genetic factors and less by environmental ones. This aspect is in agreement with basic breeding principles, indicating that plants' morphological and anatomical traits are highly heritable [29]. The differences between the three cultivated clones in some characteristics (e.g., growth habit, height, number of lateral brunches, attitude of tip) may have been due to the fact that they came from three different plants of a population, or due to a small percentage of influence of environmental factors. Acquaah [29] highlighted the variation that exists between the individual plants of a population in terms of morphological and physiological characteristics and behavior. Taking into account that the genetic background is a predominant source of phenotypical variation, our findings could also be supported by a recent study reporting high genetic diversity (H_p/H_t) among *S. scardica* populations (42.80%) sampled from mountainous areas of Greece and North Macedonia, but also within the investigated populations (57.20%) [30]. Moreover, Gratani [31] and Price et al. [32] referred to phenotypic plasticity and how environmental factors can affect the morphology and physiology of the plants. Generally, despite the fact that we investigated clones and, thus, high uniformity would be expected within the clonal lines, a relatively high variation (%CV) was estimated for some agronomic and morphological characteristics, indicating the phenotypic plasticity of plants even for highly heritable traits.

Furthermore, the studied genotypes and the cultivated population from which the clonal lines were derived expressed more developed forms with respect to certain phenotypic characteristics (plants' size/surface, elongated inflorescences, dense stems, etc.) compared to the wild-grown population (Mount Menoikio). A recent collection survey of wild *S. scardica* populations in Northern Greece indicated that the species is not competitive (unpublished data); thus, under a cultivation regime with weed control management and more favorable conditions, the changes in plant structure (plant architecture, development, organ size, etc.) and morphometric traits could be plausible. Such phenomena of morphological trait modification in the course of domestication and cultivation of MAPs have

been well documented for decades, indicating that plants' morphology in their native habitats can be environmentally influenced [33]. Nevertheless, a recent investigation of common herbaceous species validated that domestication might affect plants' size and/or biological (and physiological) processes, resulting in crops that are better than their wild progenitors [34]. It is noteworthy that *Sideritis* species naturally thrive in stony habitats, where the soil resources (water, nutrients, microorganisms, etc.) are completely different and relatively limited compared with the cultivated soil conditions.

Apart from adaptability, morphological evaluation, and agronomic evaluation, introducing an MAP biotype into cultivation requires the assessment of specific phytochemicals—"marker compounds"—determining the quality of drug products, e.g., hypericin/hyperforin and rutin in *Hypericum perforatum* L., valerenic acids in *Valeriana officinalis* L., ginkgolides and flavonoids in *Ginkgo biloba* L, etc., which exhibit significant bioactivities [35,36]. Although interesting bioactivities, such as neuroprotective potential, have been described recently for crude extracts of *S. scardica* [8,9,37], such activities have not yet been ascribed to individual metabolites (specific flavonoid or terpenoid compounds). Furthermore, Danesi et al. [38] reported a common protective activity between *S. scardica* and *Camelia sinensis* (lL.) Kuntze extracts in HepG2 cells, proposing that different classes of compounds existing in *S. scardica* may exert common activities that are possibly not limited to proanthocyanidins, which are the main compounds of *C. sinensis* associated with this activity. Several studies have summarized the qualitative polyphenolic profiles of various *Sideritis* species (including *S. scardica*) [39–42], although only few of them have reported quantitative data. In the present study, we focused on specific flavonoid compounds as qualitative markers, provided that they also serve as chemotaxonomic marker compounds for *Sideritis* species. Our analysis indicated that the SID1 and SID2 clones were superior to SID3 with respect to their contents of most of the polyphenolic compounds detected in this study. The main sources of such variation could be the genotype and/or the ratios of different plant organs (petals, sepals, bracts, leaves, and stems) in the extracted samples. A correlation between morphological traits and the biosynthesis of extracts' compounds has not been reported thus far. The variation in flavones in general, and in the identified derivatives, indicates differences in the regulation and/or expression of the involved structural genes. As all plants were grown under same conditions and in the same area, any environmental impact must have been limited. Therefore, a genetic mechanism to explain the variation can be favored and better expressed. Nevertheless, such observations and hypotheses need further detailed investigation in order to predict an accurate yield under field conditions, and to understand the genetic control of secondary pathways leading to different flavones.

Apart from polyphenolic compounds, a literature survey revealed several reports on *S. scardica* essential oil from wild-grown and cultivated plants of different origins from Turkey, Bulgaria, North Macedonia, and Greece [6,15,17,43,44]. Although their essential oil yield is extremely low (0.1–0.5%), *Sideritis* plants are characterized by an intense and pleasant aroma [45]. In the majority of published studies, a variable qualitative volatile profile of *S. scardica* essential oils has been recorded. More interestingly, the quantitative essential oil profiles varied significantly, due to the genetic variability among populations, differences in soil and environmental conditions, cultivation practices, etc. [17,46–48]. In our study, significant differences were observed in the quantitative volatile profiles of the three clones. Several factors affect the biosynthesis of essential oils: the growth stage of the plant, the effect of light quality, day length and photoperiod, ultraviolet radiation, etc. [49]. Giuliani et al. [50], reported significant differences in the essential oil compositions obtained from the leaves/stems and flower heads of *S. italica*. The production of essential oils is associated with the interaction of the genetics, ontogenesis, and physiological state of the plant with environmental conditions. The climatic conditions in the particular growing season may also have a limited effect on the essential oil compounds' biosynthesis. The three clones differed in their time of bloom, with SID1 flowering the earliest (end of April–first 10 days of May) and SID 3 the latest (end of May). In the interval of these 20 days, the temperature

(5–10 degrees difference), precipitation (28.6 mm from 15 April to 10 May; 55 mm from 11 May up to the end), and light period differed and may have influenced the biosynthesis of the essential oil compounds to an extent (Supplementary Figure S3). A recent study by Hassiotis et al. [51] reported the effects of weather conditions on the essential oil quantity and quality of *Lavandula angustifolia* cultivated in two different regions of Northern Greece. In more detail, the rainfall and lower temperatures reduced the amount of linalool by almost 10%, while *cis*-ocimene accumulation decreased in the later stages of the flowering period, suggesting that the biosynthesis of lavender monoterpenes is controlled by both growth and environmental factors, including temperature, rainfall, and flowering stage. The production of monoterpenes is also affected from the level of transcription of the genes involved in monoterpene biosynthesis, which varies during different developmental stages and environmental factors [52–54]. In the present study, during the full blooming of SID1 (1–10 May), the recorded average daily minimum–maximum temperatures were 11.5–22 °C, with precipitation of 20.2 mm; for SID2's full blooming period (10–20 May), the temperatures were 12–25 °C, with precipitation of 7.1 mm; and for SID3's full blooming period (20–31 May) the temperatures were 15–25 °C, with precipitation of 47.9 mm (Supplementary Figure S3). Additionally, taking into consideration the possible different ratios of plant parts (flowers, leaves, stalks) that constituted the plant material subjected to the distillation, the negligible amount of *S. scardica* essential oils, and the contradicting data from the literature concerning *S. scardica*'s essential oil profile (even from cultivated plants), it seems that the essential oil profile is not a stable chemometric trait that can describe a certain population. Currently, *Sideritis* essential oils have not yet been exploited. However specific chemotypes should be defined for various potential uses of essential oils in the future in medicine, pharmaceuticals, and cosmetic products.

5. Conclusions

This study revealed that significant phenotypic diversity may exist within the selected *S. scardica* population, which could be beneficial for future mass selection of individual plants and for further breeding evaluation (offspring evaluation, plasticity phenomena of the species, etc.). In addition, the genetic diversity within a population could also lead to chemodiversity, as according to our findings significant differences were observed among the three clones evaluated under the same growing and environmental conditions. Therefore, such a pre-breeding evaluation is necessary in order to identify elite genotypes from *S. scardica* expressing superior morphometric, agronomic, and phytochemical traits, such as SID2. Furthermore, although SID3 was characterized as less favorable for some morphometric traits (e.g., medium plant height, smaller leaf surface and perimeter), this clone presented a very good attitude for most of the morphological characteristics and in terms of agronomic traits. In particular, regarding plant growth, only this clone showed an upright habit, which is of great importance for mechanical harvesting. Thus, valorization of this genotype in future breeding programs studying the inheritance of its traits could be of great importance. Consequently, considering that mountain tea is a cross-pollinated species, SID2 and SID3 could be used not only for further study and as starting materials for breeding purposes, but also as parental lines in order to create progenies that will combine the favorable traits of both clones. These progenies, through a proper breeding program, could eventually lead to a certain cultivar or variety of *S. scardica* with desirable traits and high quantitative and qualitative yields.

Additionally, we report herein the first attempt to create a draft descriptor list for the main morphological characteristics of *S. scardica*. This attempt, combined with the fact that the morphological traits of a plant are quite easy to identify and highly heritable, could lead in the future to a reliable protocol for the description of *S. scardica*. Through this protocol, breeders will be able to characterize and categorize the *Sideritis* populations or genotypes, and to have the standards for developing *Sideritis* ideotypes targeting specific breeding programs and requirements of farmers, markets, and consumers.

References

1.　Evstatieva, L.N.; Alipieva, K.I. Conservation and Sustainable Use of Threatened Medicinal Plant *Sideritis scardica* in Bulgaria. *Acta Hortic.* **2012**, *955*, 89–92. [CrossRef]

2.　Duman, H.; Sideritis, L. *Flora of Turkey and East Aegean Islands (Supplement 2)*; University Press: Edinburgh, UK, 2000; Volume 11, pp. 201–205.

3.　Petrova, A.; Vladimirov, V. Red List of Bulgarian Vascular Plants. *Phytol. Balc.* **2008**, *15*, 63–94.

4.　Todorova, M.; Trendafilova, A. *Sideritis Scardica* Griseb., an Endemic Species of Balkan Peninsula: Traditional Uses, Cultivation, Chemical Composition, Biological Activity. *J. Ethnopharmacol.* **2014**, *152*, 256–265. [CrossRef]

5.　González-Burgos, E.; Carretero, M.E.; Gómez-Serranillos, M.P. *Sideritis* spp.: Uses, Chemical Composition and Pharmacological Activities—A Review. *J. Ethnopharmacol.* **2011**, *135*, 209–225. [CrossRef]

6.　Tadić, V.M.; Jeremic, I.; Dobric, S.; Isakovic, A.; Markovic, I.; Trajkovic, V.; Bojovic, D.; Arsic, I. Anti-Inflammatory, Gastroprotective, and Cytotoxic Effects of *Sideritis scardica* Extracts. *Planta Med.* **2012**, *78*, 415–427. [CrossRef] [PubMed]

7.　Irakli, M.; Tsifodimou, K.; Sarrou, E.; Chatzopoulou, P. Optimization Infusions Conditions for Improving Phenolic Content and Antioxidant Activity in *Sideritis scardica* Tea Using Response Surface Methodology. *J. Appl. Res. Med. Aromat. Plants* **2018**, *8*, 67–74. [CrossRef]

8.　Hofrichter, J.; Krohn, M.; Schumacher, T.; Lange, C.; Feistel, B.; Walbroel, B.; Pahnke, J. *Sideritis* spp. Extracts Enhance Memory and Learning in Alzheimer's β-Amyloidosis Mouse Models and Aged C57Bl/6 Mice. *J. Alzheimers Dis.* **2016**, *53*, 967–980. [CrossRef]

9.　Heiner, F.; Feistel, B.; Wink, M. *Sideritis scardica* Extracts Inhibit Aggregation and Toxicity of Amyloid-β in Caenorhabditis Elegans Used as a Model for Alzheimer's Disease. *PeerJ* **2018**, *6*, e4683. [CrossRef] [PubMed]

10.　Aligiannis, N.; Kalpoutzakis, E.; Chinou, I.B.; Mitakou, S.; Gikas, E.; Tsarbopoulos, A. Composition and Antimicrobial Activity of the Essential Oils of Five Taxa of *Sideritis* from Greece. *J. Agric. Food Chem.* **2001**, *49*, 811–815. [CrossRef] [PubMed]

11.　Petreska Stanoeva, J.; Stefova, M. Assay of Urinary Excretion of Polyphenols after Ingestion of a Cup of Mountain Tea (*Sideritis scardica*) Measured by HPLC-DAD-ESI-MS/MS. *J. Agric. Food Chem.* **2013**, *61*, 10488–10497. [CrossRef]

12.　Romanucci, V.; Di Fabio, G.; D'Alonzo, D.; Guaragna, A.; Scapagnini, G.; Zarrelli, A. Traditional Uses, Chemical Composition and Biological Activities of *Sideritis raeseri* Boiss. & Heldr. *J. Sci. Food Agric.* **2017**, *97*, 373–383. [CrossRef]

13.　Chrysargyris, A.; Kloukina, C.; Vassiliou, R.; Tomou, E.-M.; Skaltsa, H.; Tzortzakis, N. Cultivation Strategy to Improve Chemical Profile and Anti-Oxidant Activity of *Sideritis perfoliata* L. subsp. *perfoliata*. *Ind. Crops Prod.* **2019**, *140*, 111694. [CrossRef]

14.　Sarrou, E.; Doukidou, L.; Avramidou, E.V.; Martens, S.; Angeli, A.; Stagiopoulou, R.; Fyllas, N.M.; Tourvas, N.; Abraham, E.; Maloupa, E.; et al. Chemodiversity Is Closely Linked to Genetic and Environmental Diversity: Insights into the Endangered Populations of the Local Endemic Plant *Sideritis euboea* Heldr. of Evia Island (Greece). *J. Appl. Res. Med. Aromat. Plants* **2022**, *31*, 100426. [CrossRef]

15.　Baser, K.H.C.; Kirimer, N.; Tümen, G. Essential Oil of *Sideritis scardica* Griseb. subsp. *scardica*. *J. Essent. Oil Res.* **1997**, *9*, 205–207. [CrossRef]

16. Todorova, M.; Trendafilova, A.; Evstatieva, L.; Antonova, D. Volatile Components in *Sideritis scardica* Griseb. Cultivar. *Proc. Bulg. Acad. Sci.* **2013**, *66*, 507–512. [CrossRef]

17. Trendafilova, A.B.; Todorova, M.N.; Evstatieva, L.N.; Antonova, D.V. Variability in the Essential-Oil Composition of *Sideritis scardica* Griseb. from Native Bulgarian Populations. *Chem. Biodivers.* **2013**, *10*, 484–492. [CrossRef] [PubMed]

18. Venturella, P.; Bellino, A. Isolation and Partial Synthesis of Ent-18-Acetoxykaur-16-Ene-3β,7α,15β-Triol from *Sideritis scardica*. *Phytochemistry* **1979**, *18*, 1571–1572. [CrossRef]

19. Karapandzova, M.; Qazimi, B.; Stefkov, G.; Bačeva, K.; Stafilov, T.; Panovska, T.K.; Kulevanova, S. Chemical Characterization, Mineral Content and Radical Scavenging Activity of *Sideritis scardica* and *S. raeseri* from R. Macedonia and R. Albania. *Nat. Prod. Commun.* **2013**, *8*, 639–644. [CrossRef]

20. Petreska, J.; Stefkov, G.; Kulevanova, S.; Alipieva, K.; Bankova, V.; Stefova, M. Phenolic Compounds of Mountain Tea from the Balkans: LC/DAD/ESI/MSn Profile and Content. *Nat. Prod. Commun.* **2011**, *6*, 21–30. [CrossRef]

21. Petreska, J.; Stefova, M.; Ferreres, F.; Moreno, D.A.; Tomás-Barberán, F.A.; Stefkov, G.; Kulevanova, S.; Gil-Izquierdo, A. Potential Bioactive Phenolics of Macedonian *Sideritis* Species Used for Medicinal "Mountain Tea". *Food Chem.* **2011**, *125*, 13–20. [CrossRef]

22. Sarrou, E.; Martens, S.; Chatzopoulou, P. Metabolite Profiling and Antioxidative Activity of Sage (*Salvia fruticosa* Mill.) under the Influence of Genotype and Harvesting Period. *Ind. Crops Prod.* **2016**, *94*, 240–250. [CrossRef]

23. Skendi, A.; Irakli, M.; Chatzopoulou, P. Analysis of Phenolic Compounds in Greek Plants of Lamiaceae Family by HPLC. *J. Appl. Res. Med. Aromat. Plants* **2017**, *6*, 62–69. [CrossRef]

24. Rafique, M.Z.; Carvalho, E.; Stracke, R.; Palmieri, L.; Herrera, L.; Feller, A.; Malnoy, M.; Martens, S. Nonsense Mutation Inside Anthocyanidin Synthase Gene Controls Pigmentation in Yellow Raspberry (*Rubus idaeus* L.). *Front. Plant Sci.* **2016**, *7*, 1892. [CrossRef] [PubMed]

25. McCune, B.; Grace, J.B. *Analysis of Ecological Communities*; MjM Software Design: Gleneden Beach, OR, USA, 2002.

26. McCune, B.; Mefford, M.J. *PC-ORD Version 7. Multivariate Analysis of Ecological Data*; MjM Software: Gleneden Beach, OR, 2015.

27. Canter, P.H.; Thomas, H.; Ernst, E. Bringing Medicinal Plants into Cultivation: Opportunities and Challenges for Biotechnology. *Trends Biotechnol.* **2005**, *23*, 180–185. [CrossRef] [PubMed]

28. Sarrou, E.; Tsivelika, N.; Chatzopoulou, P.; Tsakalidis, G.; Menexes, G.; Mavromatis, A. Conventional Breeding of Greek Oregano (*Origanum vulgare* ssp. *hirtum*) and Development of Improved Cultivars for Yield Potential and Essential Oil Quality. *Euphytica* **2017**, *213*, 104. [CrossRef]

29. Acquaah, G. *Principles of Plant Genetics and Breeding*; John Wiley & Sons: Hoboken, NJ, USA, 2007.

30. Grdiša, M.; Radosavljević, I.; Liber, Z.; Stefkov, G.; Ralli, P.; Chatzopoulou, P.S.; Carović-Stanko, K.; Šatović, Z. Divergent Selection and Genetic Structure of *Sideritis scardica* Populations from Southern Balkan Peninsula as Revealed by AFLP Fingerprinting. *Sci. Rep.* **2019**, *9*, 12767. [CrossRef] [PubMed]

31. Gratani, L. Plant Phenotypic Plasticity in Response to Environmental Factors. *Adv. Bot.* **2014**, *2014*, e208747. [CrossRef]

32. Price, T.D.; Qvarnström, A.; Irwin, D.E. The Role of Phenotypic Plasticity in Driving Genetic Evolution. *Proc. R. Soc. Lond. B Biol. Sci.* **2003**, *270*, 1433–1440. [CrossRef]

33. Mathe, A. An Ecological Approach to Medicinal Plant Introduction. In *Herbs, Spices, and Medicinal Plants: Recent Advances in Botany. Horticulture, and Pharmacology*; Craker, L.E., Simon, J.E., Eds.; Oryx Press: Phoenix, Arizona, 1988; pp. 175–205.

34. Milla, R.; Matesanz, S. Growing Larger with Domestication: A Matter of Physiology, Morphology or Allocation? *Plant Biol.* **2017**, *19*, 475–483. [CrossRef]

35. Maiti, B.; Nagori, B.P.; Singh, R. Recent Trends in Herbal Drugs: A Review. *Int. J. Drug Res. Technol.* **2011**, *1*, 17–25.

36. Sarrou, E.; Giassafaki, L.-P.; Masuero, D.; Perenzoni, D.; Vizirianakis, I.S.; Irakli, M.; Chatzopoulou, P.; Martens, S. Metabolomics Assisted Fingerprint of *Hypericum perforatum* Chemotypes and Assessment of Their Cytotoxic Activity. *Food Chem. Toxicol.* **2018**, *114*, 325–333. [CrossRef] [PubMed]

37. Chalatsa, I.; Arvanitis, D.A.; Mikropoulou, E.V.; Giagini, A.; Papadopoulou-Daifoti, Z.; Aligiannis, N.; Halabalaki, M.; Tsarbopoulos, A.; Skaltsounis, L.A.; Sanoudou, D. Beneficial Effects of *Sideritis scardica* and *Cichorium spinosum* against Amyloidogenic Pathway and Tau Misprocessing in Alzheimer's Disease Neuronal Cell Culture Models. *J. Alzheimers Dis.* **2018**, *64*, 787–800. [CrossRef] [PubMed]

38. Danesi, F.; Saha, S.; Kroon, P.A.; Glibetić, M.; Konić-Ristić, A.; D'Antuono, L.F.; Bordoni, A. Bioactive-Rich *Sideritis scardica* Tea (Mountain Tea) is as Potent as *Camellia sinensis* Tea at Inducing Cellular Antioxidant Defences and Preventing Oxidative Stress. *J. Sci. Food Agric.* **2013**, *93*, 3558–3564. [CrossRef] [PubMed]

39. Janeska, B.; Stefova, M.; Alipieva, K. Assay of Flavonoid Aglycones from Species of the Genus *Sideritis* (Lamiaceae) from Macedonia with HPLC UV DAD. *Acta Pharm.* **2007**, *57*, 371–377. [CrossRef] [PubMed]

40. Alipieva, K.; Petreska, J.; Gil-Izquierdo, Á.; Stefova, M.; Evstatieva, L.; Bankova, V. Influence of the Extraction Method on the Yield of Flavonoids and Phenolics from *Sideritis* spp. (Pirin Mountain Tea). *Nat. Prod. Commun.* **2010**, *5*, 51–54. [CrossRef] [PubMed]

41. Pljevljakušić, D.; Šavikin, K.; Janković, T.; Zdunić, G.; Ristić, M.; Godjevac, D.; Konić-Ristić, A. Chemical Properties of the Cultivated *Sideritis raeseri* Boiss. & Heldr. subsp. *raeseri*. *Food Chem.* **2011**, *124*, 226–233. [CrossRef]

42. Stanoeva, J.P.; Stefova, M.; Stefkov, G.; Kulevanova, S.; Alipieva, K.; Bankova, V.; Aneva, I.; Evstatieva, L.N. Chemotaxonomic Contribution to the *Sideritis* Species Dilemma on the Balkans. *Biochem. Syst. Ecol.* **2015**, *61*, 477–487. [CrossRef]

43. Todorova, M.N.; Christov, R.C.; Evstatieva, L.N. Essential Oil Composition of Three *Sideritis* Species from Bulgaria. *J. Essent. Oil Res.* **2000**, *12*, 418–420. [CrossRef]

44. Qazimi, B.; Stefkov, G.; Karapandzova, M.; Cvetkovikj, I.; Kulevanova, S. Aroma Compounds of Mountain Tea (*Sideritis scardica* and *S. raeseri*) from Western Balkan. *Nat. Prod. Commun.* **2014**, *9*, 1369–1372. [CrossRef]

45. Żyżelewicz, D.; Kulbat-Warycha, K.; Oracz, J.; Żyżelewicz, K. Polyphenols and Other Bioactive Compounds of *Sideritis* Plants and Their Potential Biological Activity. *Molecules* **2020**, *25*, 3763. [CrossRef]

46. Kaparakou, E.H.; Daferera, D.; Kanakis, C.D.; Skotti, E.; Kokotou, M.G.; Tarantilis, P.A. Chemical Composition of the Essential Oils of Three Popular *Sideritis* Species Cultivated in Greece Using GC-MS Analysis. *Biomolecules* **2023**, *13*, 1157. [CrossRef] [PubMed]

47. Kostadinova, E.; Nikolova, D.; Alipieva, K.; Stefova, M.; Stefkov, G.; Evstatieva, L.; Matevski, V.; Bankova, V. Chemical Constituents of the Essential Oils of *Sideritis scardica* Griseb. and *Sideritis raeseri* Boiss and Heldr. from Bulgaria and Macedonia. *Nat. Prod. Res.* **2007**, *21*, 819–823. [CrossRef] [PubMed]

48. Skaltsa, H.; Tomou, E.; Kloukina, C. Essential Oil Composition of Two Greek Cultivated *Sideritis* spp. *Nat. Volatiles Essent. Oils* **2019**, *6*, 16–23.

49. Rehman, R.; Asif Hanif, M. Biosynthetic Factories of Essential Oils: The Aromatic Plants. *Nat. Prod. Chem. Res.* **2016**, *04*. [CrossRef]

50. Giuliani, C.; Maleci Bini, L.; Papa, F.; Cristalli, G.; Sagratini, G.; Vittori, S.; Lucarini, D.; Maggi, F. Glandular Trichomes and Essential Oil Composition of Endemic *Sideritis italica* (Mill.) Greuter et Burdet from Central Italy. *Chem. Biodivers.* **2011**, *8*, 2179–2194. [CrossRef] [PubMed]

51. Hassiotis, C.N.; Ntana, F.; Lazari, D.M.; Poulios, S.; Vlachonasios, K.E. Environmental and Developmental Factors Affect Essential Oil Production and Quality of *Lavandula angustifolia* during Flowering Period. *Ind. Crops Prod.* **2014**, *62*, 359–366. [CrossRef]

52. Lane, A.; Boecklemann, A.; Woronuk, G.N.; Sarker, L.; Mahmoud, S.S. A Genomics Resource for Investigating Regulation of Essential Oil Production in *Lavandula angustifolia*. *Planta* **2010**, *231*, 835–845. [CrossRef] [PubMed]

53. Zeng, X.; Liu, C.; Zheng, R.; Cai, X.; Luo, J.; Zou, J.; Wang, C. Emission and Accumulation of Monoterpene and the Key Terpene Synthase (TPS) Associated with Monoterpene Biosynthesis in *Osmanthus fragrans* Lour. *Front. Plant Sci.* **2016**, *6*, 1232. [CrossRef]

54. Maiti, S.; Mitra, A. Morphological, Physiological and Ultrastructural Changes in Flowers Explain the Spatio-Temporal Emission of Scent Volatiles in *Polianthes tuberosa* L. *Plant Cell Physiol.* **2017**, *58*, 2095–2111. [CrossRef]