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Hybrids Development between Greek *Salvia* Species and Their Drought Resistance Evaluation along with *Salvia fruticosa*, under Attapulgite-Amended Substrate

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Abstract: Aiming to obtain *Salvia* hybrids with ornamental value and high drought resistance, for xeriscaping, crossbreeding was made with Greek *Salvia* species. *S. fruticosa* and *S. officinalis* when used as seed parent were successfully crossed with *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa*, while when used as pollen parent it only succeeded between *S. fruticosa* and *S. tomentosa.* The growth of *S. fruticosa* and the four hybrids, *S. officinalis* × *S. pomifera*, *S. officinalis* × *S. tomentosa*, *S. officinalis* × *S. ringens* and *S. fruticosa* × *S. ringens*, selected for their ornamental traits, was evaluated under limited irrigation and modification of the substrate with attapulgite clay. The hybrids *S. officinalis* × *S. ringens* and *S. officinalis* × *S. tomentosa* developed a compact plant shape and most lateral shoots, desirable characteristics for potted plants and xeriscaping. All hybrids, especially *S. officinalis* × *S. pomifera* and *S. officinalis* × *S. tomentosa*, survived water stress better than *S. fruticosa*. Modification of the substrate with attapulgite, under limited irrigation, caused a decrease in the above ground/root biomass ratio in some hybrids and in *S. fruticosa* increased the dry weight of the root indicating increased drought resistance.

Keywords: drought tolerant; hybridization; lamiaceae; limited irrigation; mediterranean native ornamentals; sage hybrids; *Salvia officinalis*; *Salvia ringens*; *Salvia pomifera*; Salvia tomentosa; xeriscaping

1. Introduction

The genus *Salvia*, being the largest of the Lamiaceae family, with about 900 species, includes many species used as ornamental, culinary and/or medicinal plants [1]. *Salvia* species native in eastern Mediterranean regions are drought resistant, perennial, evergreen sub-shrubs being part of the macchia shrubland. In Greece, 30 *Salvia* taxa (species and subspecies) are found [2], two of which, *Salvia fruticosa* and *S. officinalis*, are commercially known. Greek *Salvia* species being drought resistant could be ideal plants for use as ornaments, in arid and semi-arid regions, particularly in xeriscaping. Xeriscaping, a landscape or gardening process that reduces or eliminates the need for irrigation, has gained worldwide acceptance in recent years, especially in areas where access to irrigation water is limited. Native plants are ideal for use in xeriscaping, since they have low irrigation and cultivation requirements, a great adaptability to different soil and climatic conditions, high ornamental value, as well as a contribution to the preservation of the character of the landscape [3–7]. Greek *Salvia* species could be also useful as landscape or garden plants, to promote bee-friendly landscaping, an international movement in support of combating pollinator population decline worldwide [8,9], as the natural vegetation attracts a large number and variety of species of insects—pollinators [10]. Drought resistant

Salvia species could also be valuable for use on extensive type urban green roofs [11,12], where certain species of *Salvia* with culinary or medicinal uses could simultaneously serve urban agriculture.

S. fruticosa Mill. (*S. triloba* L.), Greek sage, is a shrub found in the Central Greece, the Peloponnese and the Aegean islands (Table 1), growing mainly in bushy rocky areas, often on coastal cliffs, at altitudes 1–700 m [13,14]. The leaves of *S. fruticosa* have a high oil content. In Greece, the plant has a long tradition of use, since the antiquity, depicted in a Minoan fresco circa 1400 BCE at Knossos on the island of Crete [15] (Table 1). It is widely used for the preparation of an herbal tea (faskomilo), and *S. fruticosa* accounts for 50–95% of the dried sage sold in North America [16].

S. officinalis L. (Dalmatian sage, or common sage or sage) is a strongly aromatic shrub (Table 1) that prefers garrigue, stony pastures, scrub, rocky places, which has been naturalized in many places throughout the world [14,17]. It is found in Northern Greece and the Ionian islands (Table 1). Known since the antiquity for its medicinal and culinary properties, it is one of the most important species of the genus *Salvia* worldwide, as it is cultivated in many varieties as medicinal and ornamental.

Both the above species are used in xeriscaping, however *S. fruticosa* although it grows naturally in southern areas compared to *S. officinalis*, often it faces surviving problems that are attributed to limited water supply, particularly on extensive green roofs, to the contrary of *S. officinalis* that shows a higher drought resistance [12,18].

Table 1. Description and properties of the five Greek Salvia species used in interspecific crosses.

Salvia Species	Plant Height	Leaf	Flower	Distribution	Uses/Other Properties
Salvia fruticosa, Greek sage	up to 120 cm tall and wide	silvery, covered with hairs, often with 1–2 pairs of small lobes below the main one	lilac, pink or sometimes white (1.6–2.5 cm long) in early spring [14]	Endemic to the eastern Mediterranean, including southern Italy, North Africa and the Canary Islands. In Greece, in the Central country, the Peloponnese and the Aegean islands [13]	In Greece, traditionaly used as a medicinal, culinary and melliferous plant since the antiquity [15]. Widely used for the preparation of an herbal tea
Salvia officinalis, common sage	up to 60 cm tall and wide	greenish above but white felted beneath, rough, oblong to elliptical, margin finely toothed	violet-blue, pink or white (2.0–3.5 cm long) in May–July [14,17]	Widespread on the Apennines and eastern Adriatic coast [14,17]. In North and Eastern Greece and in the Ionian islands	Medicinal and culinary use, since the antiquity and medieval. Most important Salvia species worldwide, cultivated in many varieties as medicinal and ornamental
Salvia pomifera ssp. *pomifera*	up to 100 cm tall and wide	grayish, hairy, oblong to linear-oblong, rounded or subcordate at base	pink or violet in spring to early summer of intense color on elegantly curving inflorescences [19]	Grows in Crete and in the Peloponnese [13]	The leaves are used in cooking and are rich in essential oil valuable in food industry [20]
Salvia ringens	up to 30 cm tall and wide	dark green, pinnatisect or pinnate with 3–6 pairs of small lateral segments, appressed-hairy	tall (60 cm), branching flowering stems with 2–4 large (about 3.8 cm long) violet-blue flowers during late spring through summer [17]	South and Eastern parts of Balkan Peninsula, including North and Central Greece [17]	A hardy herbaceous plant resistant to low temperatures. Not used as a medicinal or culinary herb
Salvia tomentosa, Balsamic Sage	up to 80 cm tall and wide	grey-green with a rounded or heart-shaped base	usually violet with reddish-brown calyces in late spring or early summer [14,21]	Southern Europe (mostly Balkan Peninsula and Crimea) and part of Western Asia (Anatolia and Near East) [22]. In Greece: North-Eastern and Eastern Aegean Islands [2]	Drought tolerance. Not used as a medicinal or culinary herb.

Other perennial *Salvia* species native in Greece with interesting morphological characteristics include *S. pomifera* spp. *pomifera*, *S. ringens* and *S. tomentosa* (Table 1).

Salvia pomifera is an endemic species of the Eastern Mediterranean, existing in South Greece and the Aegean islands and the subspecies *S. pomifera* ssp. *pomifera* (Table 1) grows in dry, sunny places with phrygian vegetation and on rocky hillsides in Crete and in the Peloponnese [13,19]. Its leaves are rich in essential oil valuable in food industry [20].

Salvia ringens Sibth. & Sm. is a hardy low vegetation herbaceous plant (Table 1) spreading north to the highlands of Macedonia and Epirus, in Mount Olympus at altitudes up to 1900 m and in Central Greece, in areas with macchia vegetation, forest glades and streams between 490 m and 1300 m. It is resistant to low temperatures.

Salvia tomentosa Miller (*S. grandiflora*), Balsamic Sage (Table 1), is similar to *S. officinalis*, found in areas of macchia vegetation and on limestone slopes [21,22], in the North-Eastern and Central Greece and the North-Eastern and Eastern Aegean Islands [2].

The floriculture industry is constantly seeking to introduce new varieties to the market. Thus, hybrids between the above Greek *Salvia* species could exploit diversity of leaf shape, aroma, flower color and inflorescence shape of all species (Table 1), abundant early flowering of *S. fruticosa*, low compact plant shape and large flowers of *S. ringens*, adaptability to wet conditions and resistance to low temperatures of *S. tomentosa*, drought and cold resistance of *S. officinalis*, and provide new products with interesting characteristics either as potted or landscape plants. Artificial hybrids between *S. fruticosa* and *S. officinalis* are known and commercially exploited [23], while evidence for natural hybridization between them was also reported [24]. Low crossability has been reported for *S. tomentosa* with *S. officinalis* and *S. fruticosa* [25].

Attapulgite clay is a mixture of clay and non-clay minerals with its primary clay mineral being Palygorskite, a hydrous magnesium aluminosilicate. It is an inexpensive and readily available material that is widely used as a carrier due to its large specific surface area and high thermal stability [26,27]. As it can absorb heavy metals there have been reports on its use on wastewater treatment and for soil remediation [28–31]. In addition, attalulgite has been found to promote root length of *Brassica chinesis* [32] and enhance drought tolerance of sweet potato under water stress [33], when added in the substrate.

In order to introduce new drought-resistant species in the floricultural industry intended for use in the urban landscape of arid/semiarid areas, in the present study the possibility of interspecific crossbreeding of five Greek sage species with interesting floricultural characteristics and drought resistance was studied. Then, the growth of the produced hybrids that showed interesting ornamental traits and of *S. fruticosa* was tested under water stress and substrate modified with attapulgite. The latter was tested for its effectiveness in promoting plant growth under limited water supply.

2. Materials and Methods

2.1. Mother Plant Growth and Pollination Conditions

Five *Salvia* species native to Greece, i.e., *S. fruticosa*, *S. officinalis*, *S. pomifera* ssp *pomifera*, *S. ringens* and *S. tomentosa*, were chosen to incorporate a wide range of growth habit, flower color, time and duration of flowering, leaf aroma, as well as cold and drought resistance (Table 1). One genotype per species was used, obtained from cuttings collected from mature native plants grown in selected regions in Greece with high genetic variability. All plants were potted singly and grown in a greenhouse of the company Kalantzis Plants (Marathon, Attica, Greece) from October 2018 through March 2019, as well as from October 2019 through March 2020, where a minimum temperature of 18 °C was maintained. From April through September 2019 and 2020, all plants were transferred to an insect-enclosed net greenhouse for the crosses. Plants began to bloom from March and flowered simultaneously up May. In the year 2019 *S. fruticosa* plants did not flower.

Clones of *S. fruticosa* and *S. officinalis* were crossed with the clones of the other three species, *S. pomifera*, *S. ringens* and *S. tomentosa*, and each clone was used both as a pollen and seed parent for all crossings, following the methodology suggested by Tychonievich and Warner [34] for other *Salvia* species. For each interspecific cross combination, at least 100 flowers were pollinated each year. Pollinations were conducted from April through May 2019 during the morning hours and replicated during the same period in 2020. Seed parent flowers were emasculated the day before anthesis. Once the style had fully elongated, pollen was applied to the stigma directly from the dehiscing anther of the pollen parent. Pollinated flowers were observed for complete seed development. *Salvia* floral morphology is such that the developing seeds are exposed to view, and any growth of the developing seed is easily observed. Crosses were rated successful if mature, viable seed was produced. To determine the level of self-fertility of *S. fruticosa* and *S. officinalis* 50 flowers were self-pollinated as described for the crosspollinations, although without emasculation. After pollination, seeds were observed and harvested as described for the interspecific crosses. All mature seeds produced by success full crosses were harvested 4-6 weeks after crossings and stored in paper bags at room temperature for one month. All seeds were then sown under intermittent mist with bottom heat to observe germination, excepting seeds from second year crosses of *S. officinalis*, as a sufficient number of hybrids had produced from the first year.

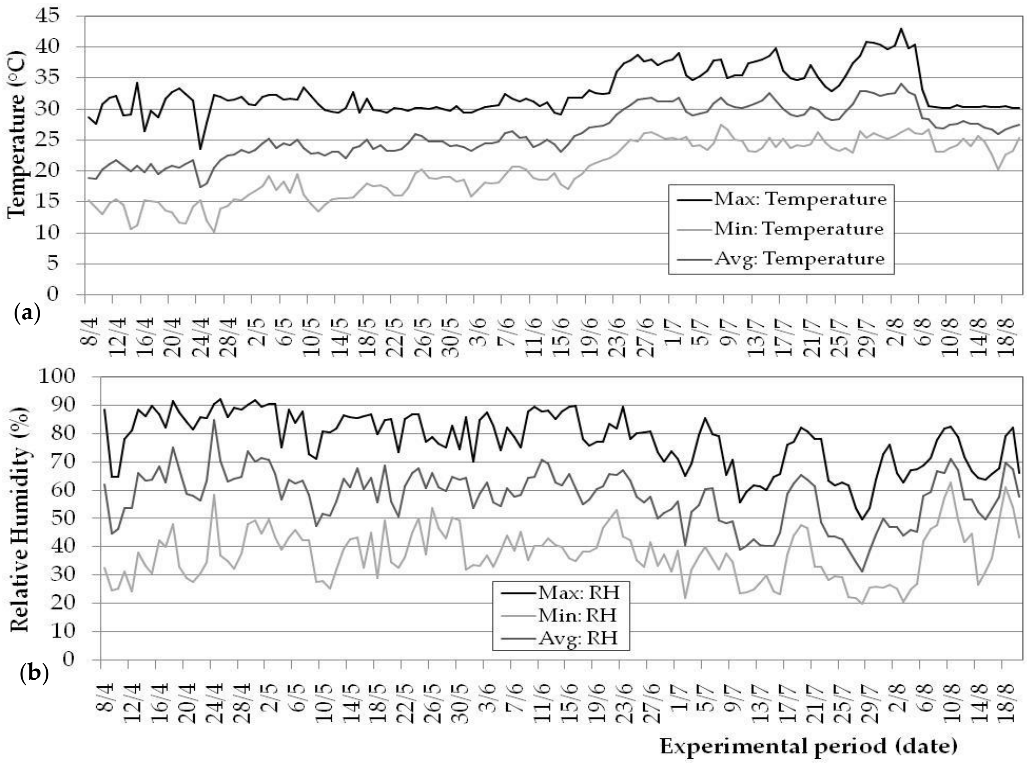
Following, seedlings were grown in the greenhouse. Besides, seeds of self-crosses were sown to estimate number of seedling production, but seedlings were not grown further. Following, all hybrids were reproduced through stem cuttings and were maintained in the greenhouse. Although evaluation of hybrids was in an initial stage, four hybrids with desirable characteristics, from different crossings, were selected for further experimentation, in the present study, concerning their resistance to drought.

2.2. Hybrids Growth

For the establishment of the experiment to test growth and drought resistance of *Salvia* hybrids, rooted cuttings of four selected hybrids of Greek sage species, produced by the crosses *S. officinalis* × *S. pomifera*, *S. officinalis* × *S. ringens*, *S. officinalis* × *S. tomentosa* and *S. fruticosa* × *S. ringens*, were used. Terminal cuttings, about 10 cm long, were collected in March 2021 from 8-month old mother plants, produced by the company Kalantzis Plants and maintained in the greenhouse (37°58′53.94″ N, 23°42′25.01″ E) at the Agricultural University of Athens. Cutting base was treated with rooting powder Rhizopon (0.5% *w*/*w* IBA in talcum, PHYTORGAN SA, Kifisia, Greece) and placed for rooting on a peat (TS 2, White peat potting substrate with adjusted pH up to 5.5 to 6.5, Klasmann-Delimann Gmbh, Geeste, Germany) and perlite (particles diameter 1 to 5 mm, Perloflor, ISOCON S.A., Athens, Greece) mixture 1:1 (*v*/*v*), in the mist (spraying 15 s per 30 min; substrate temperature 22 °C) for two weeks, followed by transfer on a light shaded location of the greenhouse for another 2 weeks.

In early April, four-week-old rooted cuttings were transplanted singly in plastic pots, 14 cm in diameter, which contained 1 L of peat-perlite mixture 2:1 (*v*/*v*), supplemented or not with 25 g/L attapulgite (AGLEV®SI 200, GEOHELLAS, Greece) and were placed in the greenhouse. Climatic conditions during the experiment are shown in Scheme 1. Physical characteristics of attapulgite were moisture 10%, granulometry 0.25–1.18 mm and density 0.72 g/cm^3. Its chemical composition was SiO_2 55.9%, $A_{12}O_3$ 6.92%, MgO 12.95%, Fe_2O_3 11.9%, CaO 0.32%, Na_2O 0.1 % and K_2O 0.43%. Plants were fertilized monthly with 2 g/L water soluble fertilizer (20-20-20 plus, HUMOFERT, Metamorfosi, Greece). In each pot, 100 mL of fertilizer was applied.

Two irrigation frequencies were applied, i.e., (i) when the moisture content of the substrate was 20–23% *v*/*v* (normal irrigation) and (ii) when the moisture content of the substrate was 8–13% *v*/*v* (sparse irrigation). In the first month of cultivation, the plants under normal irrigation were irrigated every 3–4 days and under sparse irrigation every 5 days, while in the following months every 2 and 3–4 days, respectively. Irrigation frequencies were determined during the first two weeks of cultivation, by daily measurements of the substrate moisture. Symptoms of wilting (leaf wilting and slight twisting at the top) of 1/3 of the plants and dry substrate surface in all pots determined the sparse irrigation frequency, while the dry substrate surface in 1/3 of the pots determined the normal irrigation frequency. Substrate moisture measurements (% v/v) were obtained using a handheld time domain reflectometry moisture meter (HH2; Delta-T devices, Cambridge, UK) with a dielectric soil moisture sensor (WET-2; Delta-T devices) inserted from the surface that measured 65 mm in depth and 45 mm in width.



Scheme 1. Climatic conditions, i.e., temperature (**a**) and relative humidity (**b**) inside the glass greenhouse where the experiment was conducted, during the experimental period from 8 April 2021 until 20 August 2021.

Plant growth was evaluated after a three-month culture, from April to July 2021, recording plant height (from pot rim to the highest plant point), lateral shoot number and length, and fresh and dry weight of the above ground part and the root system of the plant.

2.3. *Salvia fruticosa* Growth

Rooted cuttings of *S. fruticosa* were also produced following the same procedure as for hybrid cuttings, but they were collected one month later from mother plants almost 3 years old. In mid-May, four-week-old rooted cuttings were transplanted singly in plastic pots, 14 cm in diameter, which contained 1 L of peat-perlite mixture 2:1 (v/v), supplemented with various concentrations of attapulgite, 0 (control) or 6.25 or 12.5 or 25 g/L, that were equivalent to 0 or 1 or 2 or 4 teaspoons per pot, respectively. They were placed in the greenhouse too, under the climatic conditions presented in Scheme 1. Plants of *S. fruticosa* received the same fertlization and irrrigation as the hybrids. Total number of irrigations was 38 and 27 in normal and sparse irrigation, respectively. This experiment lasted from May to August 2021, and the above mentioned growth parameters for hybrids were estimated as well.

Based on personal observation that hybrids grown in the substrate that contained attapulgite seemed less stressed during a heat wave than those grown without attapulgite, extra measurements were taken in the *S. fruticosa* experiment (that was terminated one month later than the experiment with the hybrids) regarding the determination of substrate moisture during different climatic conditions, in order to reveal the rate and the level of moisture reduction in the root area. So, substrate moisture (%) was recorded using a handheld time domain reflectometry moisture meter (see above), during a heat wave, from 2 to 3 August 2021, as well as during a period with normal summer temperatures, from 12 to 15 August 2021 (Scheme 1). During the first period, plants were irrigated normally every day and sparsely every second day, while during the second period irrigation frequency was every second day and every four days for normal and sparse irrigation, respectively.

2.4. Statistical Analysis

The completely randomized design was used. The significance of the results was tested by one- or two- or three-way analysis of variance (ANOVA) and treatment means were compared by Student's *t* test at $p \leq 0.05$ (JMP 13.0 software, SAS Institute Inc., Cary, NC, USA, 2013).

3. Results

3.1. Interspecific Crosses and Hybrids Production

Interspecific crosses of *S. officinalis* with *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa*, which were conducted during two successive years, in spring 2019 and 2020, showed that crosses were successful only when *S. officinalis* was used as seed parent and unsuccessful when it was used as pollen parent. Interspecific crossability was successful at much lower percentage (lower that 10%) compared to self-crossing of *S. officinalis* that was quite high (80%) (Table 2). Regarding interspecific crosses of *S. fruticosa* with *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa*, when *S. fruticosa* was used as seed parent, crosses with *S. ringens* and *S. tomentosa* were more successful than those with *S. pomifera.* When *S. fruticosa* was used as pollen parent, only the cross *S. tomentosa* × *S. fruticosa* was feasible and successful at quite high percentage (53%). Self-crossing of *S. fruticosa* was the most successful (92%) (Table 2).

Table 2. Interspecific crosses and self-pollinations of Greek sage species (average data of 2019 and 2020 crosses).

Cross	Pollinations Number	Successful Crosses (%)	Total Seed Number	Seedling Number *
S. officinalis × self	50	80	40	29
S. officinalis × *S. ringens*	502	5.7	27	7
S. officinalis × *S. pomifera*	531	5.6	30	6
S. officinalis × *S. tomentosa*	389	6.4	24	5
S. ringens × *S. officinalis*	200	0	0	0
S. pomifera × *S. officinalis*	200	0	0	0
S. tomentosa × *S. officinalis*	200	0	0	0
S. fruticosa × self	50	92	48	39
S. fruticosa × *S. ringens*	100	19	19	4
S. fruticosa × *S. pomifera*	100	8	8	1
S. fruticosa × *S. tomentosa*	100	28	28	0
S. ringens × *S. fruticosa*	100	0	0	0
S. pomifera × *S. fruticosa*	100	0	0	0
S. tomentosa × *S. fruticosa*	100	53	160	11

Seedling number *: results of one year.

During the two years of crossings, more than 30 hybrids were produced, which were transferred to the greenhouse facilities in order to be evaluated in terms of their development and the desired characteristics (easy propagation by cuttings and cultivation inside a greenhouse, fast growth, early and intense flowering, small plant size). Four of the hybrids stood out during the initial evaluation stages. The following basic characteristics give to the selected hybrids particular ornamental value (detailed data are not presented in the present article):

S. officinalis × S. *pomifera* produces a few long lateral shoots and seems to have intermediate characteristics of its parents in plant height, leaf color and shape, as well as in leaf aroma. Both of its parents are strongly aromatic plants and this hybrid has a pleasant distinctive aroma, which is slightly closer to the aroma of *S. pomifera*. It didn't flower during the first year.

S. officinalis × *S. ringens* has inherited from *S. ringens* the segmented leaves and the long flowering stems. It is taller than *S. ringens* and produces many lateral shoots, while its segmented leaves are unique and particularly decorative, but they have very light aroma, as *S. ringens* does. Flowering stems are about 40 cm long with more flowers than those of *S. ringens*. Flower color is light violet-blue.

S. officinalis × *S. tomentosa* is a compact plant that produces numerous lateral shoots with grey-green leaves smaller than those of *S. officinalis*. It also forms many flowering stems with light pink flowers that look more like those of *S. officinalis*. Moreover, it has a lighter aroma than that of *S. officinalis*.

S. fruticosa × *S. ringens* looks like *S. fruticosa* in height and shape, but it has intensely hairy segmented leaves, which have very light aroma like that of *S. ringens*. Its flowering stems are longer (about 80 cm) than those of *S. fruticosa*, while flowers are more sparsely arranged, characteristics inherited from *S. ringens*. Flower color is light purple.

3.2. Hybrids Growth

All *Salvia* hybrids grew successfully in all treatments during the three months of the growing period. Three-way ANOVA at the end of the culture period (Table 3) showed interaction of the three experimental factors only in the lateral shoot mean and total length. The type of hybrid affected strongly plant height (main shoot length), lateral shoot number and above ground fresh and dry weight. *S. officinalis* × *S. ringens* and *S. officinalis* × *S. tomentosa* were the shortest hybrids with large number of laterals, while *S. officinalis* × *S. pomifera* had long main shoot and the smallest number of laterals and *S. fruticosa* × *S. ringens* equally long main shoot but large number of laterals that had the smallest length. Sparse irrigation reduced plant height and above ground fresh and dry weight and attapulgite amendment to the substrate reduced lateral shoot number, above ground fresh and dry weight, roots fresh weight and the ratio above ground/roots fresh weight (Table 3).

Hybrid growth was then examined comparatively regarding the effect of hybrid type and irrigation frequency separately in each substrate (two-way anova, Tables 4 and 5).

3.2.1. Peat-Perlite 2:1 (v/v)

Evaluating the drought resistance of hybrids when grown in the substrate without attapulgite (Table 4), it was found that both *S. ringens* hybrids although had root fresh and dry weight reduced under sparse irrigation, none growth parameter concerning foliage was affected, and thus the above ground/root fresh and dry weight ratio was increased, having the highest value in *S. officinalis* × *S. ringens* among all hybrids. To the contrary, *S. officinalis* × *S. pomifera* and *S. officinalis* × *S. tomentosa* had some restriction of shoot elongation under sparse irrigation; however, it was not expressed in the above ground fresh and dry weight and their root dry weight was not affected. Under normal irrigation, *S. fruticosa* × *S. ringens* had the highest of all hybrids root fresh and dry weight, and along with *S. officinalis* × *S. pomifera* presented the lowest above ground/root dry weight ratio (Table 4 and Figure 1).

Table 3. The effect of the experimental factors, i.e., hybrid type (*S. officinalis* × *S. pomifera*, *S. officinalis* × *S. ringens*, *S. officinalis* × *S. tomentosa*, *S. fruticosa* × *S. ringens*), irrigation frequency (normal, sparse) and substrate type (with 25 g/L attapulgite, without attapulgite) on above ground and root system growth parameters of sage interspecific hybrids.

3-Way ANOVA	Plant Height (cm)	Lateral Shoot Number	Lateral Shoot Mean Length (cm)	Lateral Shoot Total Length (cm)	Above Ground f.w. (g)	Roots f.w. (g)	Above Ground f.w./Roots f.w.	Above Ground d.w. (g)	Roots d.w. (g)	Above Ground d.w./Roots d.w.
S. officinalis × *S. pomifera*	32.8 a [z]	4.4 b	11.5	49.4	22.3 c	15.4	1.5	8.4 c	3.5	2.5
S. officinalis × *S. ringens*	27.0 b	7.7 a	10.1	73.2	31.4 a	10.4	3.2	11.6 a	3.0	4.2
S. officinalis × *S. tomentosa*	26.9 b	8.4 a	9.6	81.6	27.8 b	10.0	2.9	9.3 b	2.6	3.6
S. fruticosa × *S. ringens*	32.4 a	8.5 a	8.9	76.6	27.5 b	15.5	1.9	9.6 b	3.7	2.7
without attapulgite	30.0 a	7.7 a	10.2	75.9	28.1 a	13.2 a	2.5 a	10.1 a	3.3 a	3.5
with 25 g/L attapulgite	29.5 a	6.8 b	9.9	64.5	26.4 b	12.4 b	2.3 b	9.4 b	3.1 a	3.0
normal irrigation	31.9 a	7.6 a	10.4	75.7	28.5 a	13.5	2.4	10.3 a	3.4	3.3
sparse irrigation	27.6 b	6.9 a	9.6	64.6	25.9 b	12.1	2.4	9.2 b	3.0	3.2
				Significance [§]						
F_{hybrid}	**	**	-	-	**	-	-	**	-	-
$F_{\text{attapulgite}}$	NS	*	-	-	*	*	*	**	NS	-
$F_{\text{irrigation}}$	**	NS	-	-	**	-	-	**	-	-
$F_{\text{hybrid x attapulgite}}$	NS	NS	-	-	NS	NS	NS	NS	NS	NS
$F_{\text{hybrid x irrigation}}$	NS	NS	-	-	NS	**	**	NS	**	**
$F_{\text{attapulgite x irrigation}}$	NS	NS	-	-	NS	NS	NS	NS	NS	**
$F_{\text{hybrid x attapulgite x irrigation}}$	NS	NS	*	*	NS	NS	NS	NS	NS	NS

[z] Mean comparison in columns within each main factor with Student's t test at $p \leq 0.05$; means followed by the same letter are not significantly different at $p \leq 0.05$. [§] NS or * or **, non-significant at $p \leq 0.05$ or significant at $p \leq 0.05$ or $p \leq 0.01$, respectively.

Table 4. Comparative evaluation of growth of four new interspecific hybrids of Greek sage species regarding resistance to water deficiency, after three months of greenhouse culture on peat: perlite 2:1 (v/v).

Hybrid Type	Irrigation Frequency	Plant Height (cm)	Lateral Shoot Number	Lateral Shoot Mean Length (cm)	Lateral Shoot Total Length (cm)	Above Ground f.w. (g)	Root f.w. (g)	Above Ground f.w./Roots f.w.	Above Ground d.w. (g)	Root d.w. (g)	Above Ground d.w./Root d.w.
S. officinalis × *S. pomifera*	normal	36.9 a [†]	4.6 c	12.8 a	53.8 cd	24.7 cd	14.2 bc	1.8 de	9.4 bc	3.5 b	2.7 cde
	sparse	30.2 bc	4.3 c	10.2 b	44.4 d	20.7 d	15.0 b	1.4 e	8.1 c	3.2 b	2.5 e
S. officinalis × *S. ringens*	normal	27.5 cd	9.0 ab	10.1 b	87.4 ab	34.6 a	12.9 cd	2.7 c	12.9 a	3.4 b	3.8 b
	sparse	27.0 cd	7.6 b	10.6 b	75.0 bc	33.0 ab	8.3 f	4.1 a	12.1 a	2.4 c	5.8 a
S. officinalis × *S. tomentosa*	normal	30.1 bc	10.3 a	10.2 b	105.1 a	29.0 bc	8.5 f	3.5 b	9.4 bc	2.6 c	3.7 b
	sparse	24.6 d	8.1 b	9.1 b	73.5 bc	26.2 c	10.9 e	2.5 c	9.0 bc	2.6 c	3.6 bcd
S. fruticosa × *S. ringens*	normal	32.8 ab	8.7 ab	8.7 b	78.4 b	28.5 c	17.3 a	1.7 e	10.4 b	4.1 a	2.6 de
	sparse	30.7 bc	9.2 ab	9.8 b	89.4 ab	27.7 c	12.3 de	2.3 cd	9.8 b	3.2 b	3.1 bcde
					Significance [§]						
F_{hybrid}		**	**	*	**	**	-	**	**	-	-
$F_{irrigation}$		**	NS	NS	NS	*	-	NS	**	-	-
$F_{interaction}$		NS	NS	NS	NS	NS	**	NS	NS	*	*
$F_{\text{one-way ANOVA}}$		**	**	*	**	**	**	**	**	**	**

[†] Mean values ($n = 10$) in each column followed by the same lower-case letter do not differ significantly at $p \leq 0.05$ by Student's t test. [§] NS or * or **, non-significant at $p \leq 0.05$ or significant at $p \leq 0.05$ or $p \leq 0.01$, respectively.

Table 5. Comparative evaluation of growth of four new interspecific hybrids of Greek sage species regarding resistance to water deficiency, after three months of greenhouse culture on peat: perlite 2:1 (*v*/*v*) with 25 g/L attapulgite.

Hybrid Type	Irrigation Frequency	Plant Height (cm)	Lateral Shoot Number	Lateral Shoot Mean Length (cm)	Lateral Shoot Total Length (cm)	Above Ground f.w. (g)	Roots f.w. (g)	Above Ground f.w./Roots f.w.	Above Ground d.w. (g)	Roots d.w. (g)	Above Ground d.w./Roots d.w.
S. officinalis × *S. pomifera*	normal	36.0 a [†]	4.2 c	11.6 a	48.3 c	22.3 de	15.5 bc	1.5 de	8.4 cd	3.5 b	2.4 d
	sparse	28.0 cd	4.5 c	11.6 a	50.9 c	21.3 e	16.7 ab	1.3 e	7.8 d	3.5 b	2.2 d
S. officinalis × *S. ringens*	normal	29.1 bcd	8.0 ab	10.8 ab	78.2 a	33.0 a	11.8 de	2.9 ab	12.1 a	3.4 b	3.8 ab
	sparse	24.3 d	6.2 bc	8.9 bc	52.0 c	25.1 cd	8.4 g	3.2 a	9.3 bc	2.7 c	3.6 ab
S. officinalis × *S. tomentosa*	normal	28.3 cd	7.4 ab	9.9 ab	72.4 ab	29.1 b	9.4 fg	3.4 a	10.0 b	2.5 c	4.1 a
	sparse	24.7 d	7.9 ab	9.3 bc	75.4 ab	26.7 bc	11.2 ef	2.4 bc	9.0 bcd	2.9 c	3.2 bc
S. fruticosa × *S. ringens*	normal	34.6 ab	8.8 a	9.3 bc	81.9 a	26.9 bc	18.6 a	1.5 de	9.5 bc	4.2 a	2.3 d
	sparse	31.3 abc	7.4 ab	7.6 c	56.6 bc	26.7 bc	13.8 cd	2.0 cd	8.7 bcd	3.4 b	2.6 cd
					Significance [§]						
F_{hybrid}		**	**	**	-	-	-	-	**	-	-
$F_{\text{irrigation}}$		**	NS	*	-	-	-	-	**	-	-
$F_{\text{interaction}}$		NS	NS	NS	*	**	**	**	NS	**	*
$F_{\text{one-way ANOVA}}$		**	**	**	**	**	**	**	**	**	**

[†] Mean values (n = 10) in each column followed by the same lower-case letter do not differ significantly at $p \leq 0.05$ by Student's t test. [§] NS or * or **, non-significant at $p \leq 0.05$ or significant at $p \leq 0.05$ or $p \leq 0.01$, respectively.

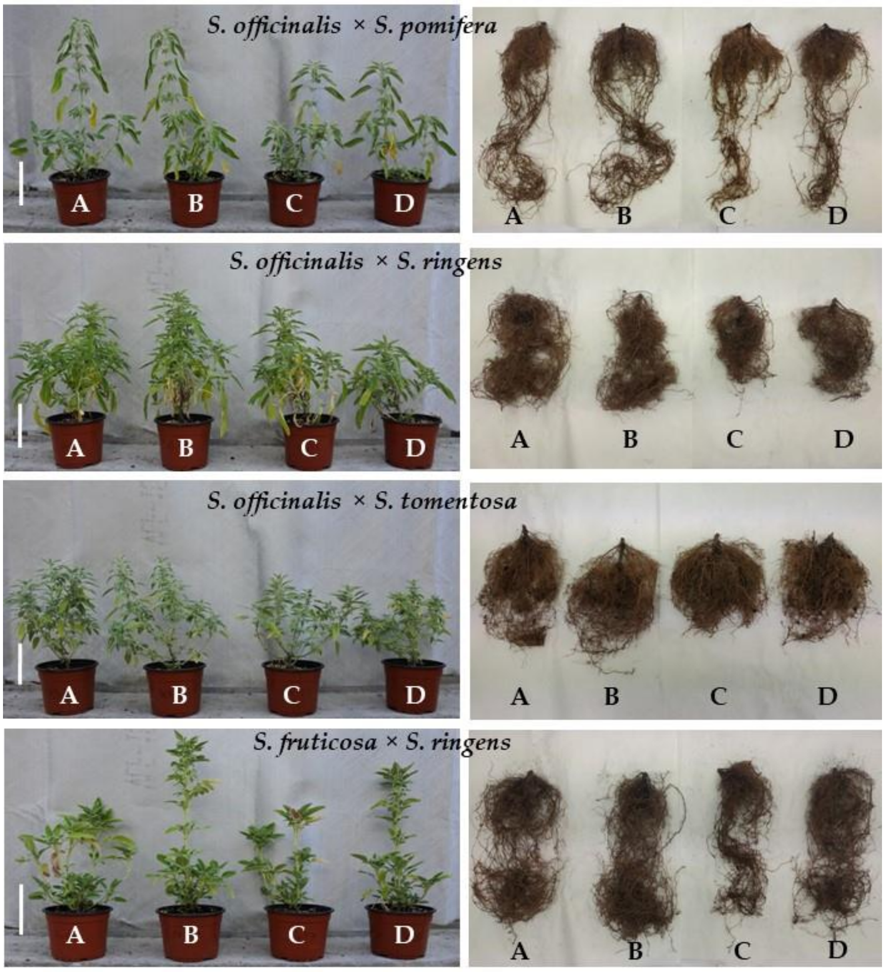


Figure 1. Typical above ground and root system of the marked hybrids of Greek sage species, after having grown for three months in greenhouse conditions (A: without and B: with 25 g/L attapulgite/normal irrigation, C: without and D: with 25 g/L attapulgite/sparse irrigation). Size bars = 10.0 cm.

3.2.2. Peat-Perlite 2:1 (*v*/*v*) plus 25 g/L Attapulgite

When attapulgite was used in the substrate (Table 5), under sparse irrigation, both *S. ringens* hybrids had root fresh and dry weight, as well as total lateral shoot length reduced and *S. officinalis* × *S. ringens* had the above ground fresh weight reduced, as well. *S. officinalis* × *S. pomifera* presented similar above ground and root growth as those in the substrate without attapulgite (Tables 4 and 5 and Figure 1), while *S. officinalis* × *S. tomentosa* had none of growth parameters measured reduced when sparse irrigation was applied, apart from the ratios above ground/root fresh and dry weight. Under normal irrigation, *S. fruticosa* × *S. ringens* had the highest of all hybrids root fresh and dry weight, and along with *S. officinalis* × *S. pomifera* presented the lowest above ground/root dry weight ratio (Table 5).

3.3. *Salvia fruticosa* Growth

Studying growth of *S. fruticosa* cultivated under various concentrations of attapulgite and irrigated with the same frequencies (normal, sparse) as the hybrids, a significant number of plant loses was recorded. Plant losses reached 8% in most treatments, except for plants grown on substrates containing the two highest concentrations (12.5 and 25.0 g/L) of attapulgite and irrigated sparsely, in which losses reached 17%.

As regards the effect of the experimental factors, irrigation frequency affected all growth parameters, apart from root fresh weight, while attapulgite concentration affected only parameters concerning plant biomass (Table 6).

Plants irrigated normally were taller (not in all treatments statistically significant) and had greater axillary shoot total length, irrespectively attapulgite concentration, while there were no differences in axillary shoot number (Table 6 and Figure 2). Both fresh and with attapulgite at concentrations shown dry weight of canopy were favored by normal irrigation. However, when sparse irrigation was applied, the addition of attapulgite at 6.25 or 12.5 g/L increased canopy fresh and dry weight compared to higher attapulgite concentration (25.0 g/L) or the control. Roots fresh weight was the only parameter that was not affected by irrigation frequency and along with roots dry weight, they were greater at 25g/L attapulgite (Table 6).

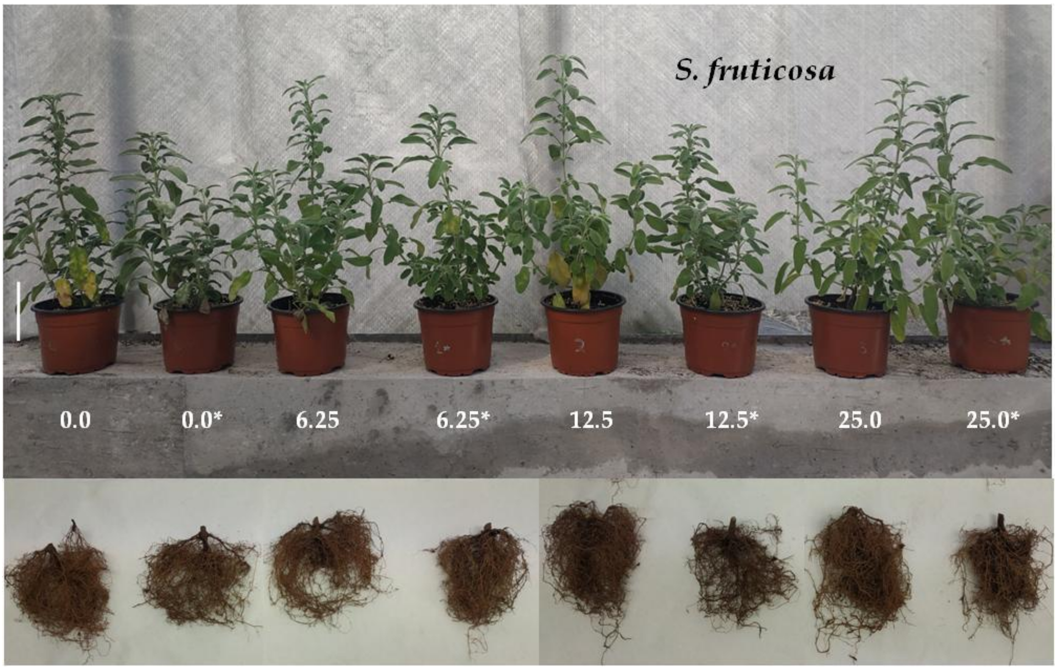


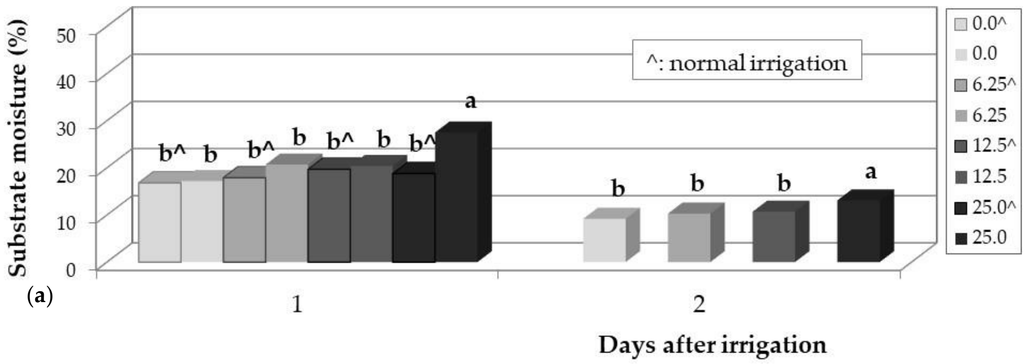
Figure 2. Typical canopy and roots of *S. fruticosa*, after having grown for three months in greenhouse conditions in a peat-perlite 2:1 (v/v) substrate containing marked concentrations (g/L) of attapulgite (* sparse irrigation). Size bars = 10.0 cm.

Studying the effect of attapulgite on the substrate moisture under two different climatic conditions showed that during a heat wave, in the beginning of August 2021 (Figure 1), when plants were irrigated every day (normal irrigation) and every second day (sparse irrigation), the substrates that contained attapulgite had relatively higher moisture percent compared to the control, but this was statistically significant only at 25 g/L attapulgite (Scheme 2a). During a period with normal summer temperatures, in the middle of August 2021 (Scheme 1), plants were irrigated every second day (normal irrigation) and every four days (sparse irrigation). At the first day after irrigation, the moisture content of substrates irrigated normally was higher than those irrigated sparsely, especially in the substrates with 0 or 6.25 g/L attapulgite, while at the second day there were no differences among treatments (Scheme 2b). During the following two days, recording of moisture continued only in the substrates that were sparsely irrigated and showed that the substrates with 12.5 or 25 g/L attapulgite have preserved higher moisture percent than those with 0 or 6.25 g/L attapulgite (Scheme 2b).

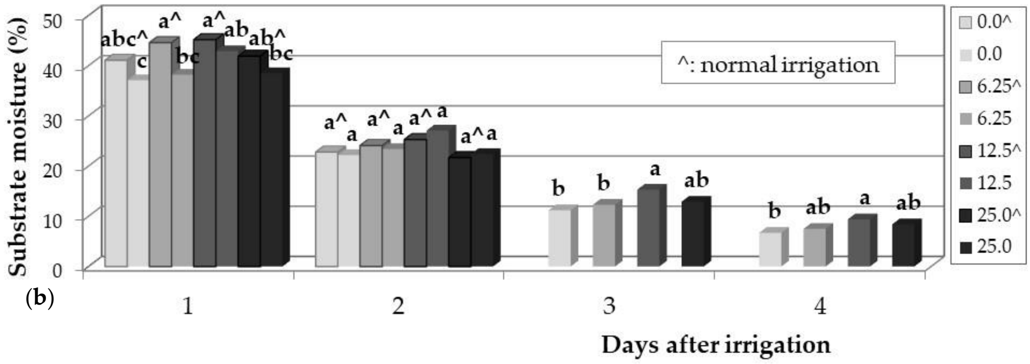
Table 6. Effect of attapulgite concentration (g/L) and irrigation frequency on growth of *Salvia fruticosa*, after three months of greenhouse culture on peat: perlite 2:1 (*v*/*v*).

Attapulgite Concentration (g/L)	Irrigation Frequency	Plant Height (cm)	Lateral Shoot Number	Lateral Shoot Mean Length (cm)	Lateral Shoot Total Length (cm)	Above Ground f.w. (g)	Roots f.w. (g)	Above Ground f.w./Roots f.w.	Above Ground d.w. (g)	Roots d.w. (g)	Above Ground d.w./Roots d.w.
0.0	normal	36.1 ab †	7.0 a	15.8 abc	108.4 a	36.4 ab	9.2 d	4.1 ab	10.5 a	2.6 bc	4.2 ab
	sparse	33.8 abc	6.1 a	14.3 c	85.9 b	28.9 d	9.2 d	3.2 cd	6.7 c	2.1 e	3.4 cd
6.25	normal	36.8 a	7.2 a	16.5 abc	112.8 a	39.7 a	9.5 cd	4.4 a	11.4 a	2.4 cde	4.8 a
	sparse	32.6 bc	6.6 a	13.9 c	89.7 b	33.3 bc	9.5 cd	3.7 bc	8.4 b	2.1 de	4.2 ab
12.5	normal	37.0 a	6.5 a	18.4 a	112.1 a	36.8 ab	10.4 bcd	3.6 bc	10.8 a	2.6 abc	4.2 ab
	sparse	33.3 abc	5.8 a	15.3 bc	87.9 b	30.5 cd	11.5 abc	2.8 de	7.8 b	2.5 bcd	3.2 cd
25.0	normal	37.3 a	6.6 a	17.4 ab	114.5 a	37.7 a	12.1 ab	3.3 cd	10.8 a	3.0 a	3.7 bc
	sparse	31.6 c	5.3 a	14.2 c	74.8 b	28.0 d	12.8 a	2.3 e	7.5 bc	2.9 ab	2.7 d
					Significance §						
$F_{attapulgite}$		NS	NS	NS	NS	*	**	**	*	**	**
$F_{irrigation}$		**	*	**	**	**	NS	**	**	*	**
$F_{interaction}$		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$F_{one\text{-}way\ ANOVA}$		*	NS	*	**	**	**	**	**	**	**

† Mean values (n = 11–12) in each column followed by the same lower-case letter do not differ significantly at $p \leq 0.05$ by Student's t test. § NS or * or **, non-significant at $p \leq 0.05$ or significant at $p \leq 0.05$ or $p \leq 0.01$, respectively.



Substrate Moisture (%)	Day 1 (2 August 2021)	Day 2 (3 August 2021)
$F_{\text{attapulgite}}$	**	-
$F_{\text{irrigation}}$	**	-
$F_{\text{interaction}}$	NS	-
$F_{\text{one-way ANOVA}}$	**	**



Substrate Moisture (%)	Day 1 (12 August 2021)	Day 2 (13 August 2021)	Day 3 (14 August 2021)	Day 4 (15 August 2021)
$F_{\text{attapulgite}}$	*	NS	-	-
$F_{\text{irrigation}}$	**	NS	-	-
$F_{\text{interaction}}$	NS	NS	-	-
$F_{\text{one-way ANOVA}}$	**	NS	*	*

Mean values (n = 12) in each day followed by the same lower-case letter do not differ significantly at $p \leq 0.05$ by Student's t test.
NS or * or **, non-significant at $p \leq 0.05$ or significant at $p \leq 0.05$ or $p \leq 0.01$, respectively.

Scheme 2. Effect of attapulgite concentration (g/L) and irrigation frequency on moisture of the substrate used for *Salvia fruticosa* cultivation under greenhouse conditions, (**a**) during a heat wave or (**b**) during a period with normal summer temperatures. (^: normal irrigation; different shades of gray indicate different concentrations of attapulgite)

3.4. Flowering

Regarding flowering, less than 40–50% of the *S. officinalis* × *S. ringens* and *S. fruticosa* × *S. ringens* plants in each treatment flowered, forming only one inflorescence (one flowering shoot) per plant, whereas more than 80% of the *S. officinalis* × *S. tomentosa* plants flowered forming about two inflorescence (two flowering shoots) per plant. No plant of *S. fruticosa* and *S. officinalis* × *S. pomifera* flowered (data not shown).

4. Discussion

4.1. Interspecific Crosses and Hybrids Production

The five Mediterranean *Salvia* species native to Greece, *S. fruticosa*, *S. officinalis*, *S. pomifera* ssp *pomifera*, *S. ringens* and *S. tomentosa*, belong to the *Salvia officinalis* group that consists of eight to ten perennial species, which are distributed in the Mediterranean Basin and the Near East [25]. In the present work, they were used for the development of new interspecific hybrids, for commercial ornamental use, and were selected to incorporate a wide range of characteristics concerning growth habit, flower color, time and duration of flowering, leaf aroma, as well as resistance to cold and drought [14,17].

Although the species from central and southern Greece (*S. fruticosa* and *S. pomifera* ssp. *pomifera*) flower earlier (April–early May) in nature compared to the other three species from northern Greece (May–early June), plants of all species flowered simultaneously in the greenhouse, from the beginning of March, due to the minimum temperature of 18 °C that was maintained in the greenhouse. This allowed us to proceed to all desired crosses.

S. fruticosa, S. officinalis and S. pomifera have the same number of chromosomes, 2n = 14 [35], while *S. tomentosa* has 2n = 16 and *S. ringens* 2n = 12 [36], although the genus *Salvia* is thought as highly tolerant of aneuploidy in its hybrids and thus different chromosome numbers in this genus are not a barrier to the formation of hybrids [34].

S. officinalis and *S. fruticosa* genotypes used showed high, to very high in the case of *S. fruticosa,* self-pollination ability, similarly to previous report [25]. Interspecific crosses of *S. officinalis* with *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa* were successful, though with very low crossability (5.6–6.4%) and only when *S. officinalis* was used as seed parent. Putievsky et al. [25] also reported extremely low crossability between *S. tomentosa* and *S. officinalis* (2%) and in their work *S. officinalis* acted as a pollen parent due to delayed flowering of *S. tomentosa*. Thus, it appears that crosses between *S. officinalis* and *S. tomentosa* are possible regardless of which species acts as the seed parent. The very low crossability probably did not allow the present work to yield hybrids when *S. officinalis* was acting as a pollen parent.

Crosses of *S. fruticosa* with *S. pomifera* ssp. *pomifera* (8% success) and *S. ringens* (19% success) succeeded only when *S. fruticosa* was used as seed parent. Only crosses of *S. fruticosa* with *S. tomentosa* were successful both when *S. fruticosa* was used as seed (28% success) or pollen parent (53% success) and with much higher crossabiltity. Previous successful crossbreeding (21%) with *S. tomentosa* acting as seed parent and *S. fruticosa* as pollen parent has been reported, where delayed flowering of *S. tomentosa* did not allow acting as pollen parent for *S. fruticosa* [25]. The tested sage species, although having low crossability in most crosses, are still closely related. They appear to be reproductively isolated primary by geographical and ecological barriers and by different flowering periods, rather than from genetic barriers [25].

A significant number of the hybrid seeds did not germinate; however, more than 30 hybrids were produced and four stood out during the early stages of evaluation for ornamental value and ease of greenhouse cultivation and were further tested in the present work for their drought resistance.

4.2. Salvia Hybrids and S. fruticosa Growth and Drought Resistance

S. officinalis × *S. ringens* was a short plant indicating that its height was affected mostly by *S. ringens* that is a low herbaceous sage, while *S. fruticosa* × *S. ringens* was a tall plant indicating predominance of *S. fruticosa* concerning this characteristic. *S. officinalis* × *S. tomentosa* unlike both the parental species was a short plant, a result that is not uncommon in hybridization, while *S. officinalis* × *S. pomifera* was a tall plant probably due to *S. pomifera*, which is the tallest sage species we used. This hybrid produced few long shoots that is not a positive horticultural trait. However, it has a very pleasant aroma. All hybrids that were produced where tested for their morphology after applying pruning too, before selecting these (four) that we used for further experimentation on drought resistance. Appling pruning at an early growth stage, which is a routine horticultural practice for ornamentals, resulted in a compact plant shape in all hybrids. In this work, we do not present in detail the selection process of hybrids; we focus on their behavior towards drought resistance.

All four *Salvia* hybrids grew successfully in all treatments contrary to *S. fruticosa* that presented 8–17% (depending on treatment) plant losses even under normal irrigation frequency that is applied in commercial floriculture greenhouses. *S. fruticosa* when cultured on an extensive green roof could not tolerate lack of irrigation for a long period [12], although in nature is a very drought resistant plant [15]. Probably the plant in nature develops deep roots that provide sufficient water, from deeper soil layers, to survive prolonged periods of drought, something that cannot happen in a pot or a green roof system.

Water stress reduced the above ground and root growth of *S. fruticosa*, while in hybrids *S. officinalis* × *S. pomifera* and *S. officinalis* × *S. tomentosa*, although it induced some restriction of shoot elongation, this was not expressed in the above ground fresh and dry weight and their root biomass was not affected, indicating a higher drought resistance compared to *S. fruticosa*. Growth is a plant response to water stress and a reliable criterion for assessing the degree of drought and drought resistance of plants. Decreases in plant height are common under drought stress [37], and as the main reason for this is considered that the lack of water leads to clogging of vascular tissue and reduction of cell elongation [38].

Water deficiency has been reported to reduce plant height and plants yield components (above ground and leaf fresh and dry weight) in *S. fruticosa* [39] and *S. officinalis* [40,41]. Regarding other Labiatae species, in *Lavandula latifolia*, *Mentha piperita* and *Thymus capitatus*, the above ground fresh weight was reduced by drought stress, whereas in *Salvia sclarea*, *Salvia lavandutifolia* and *Thymus mastichina* it remained unaffected. As regards the above ground dry weight, only in *L. latifolia* there was a significant reduction under water deficit conditions [42]. The effect of water stress in roots was not estimated in none of the abovementioned studies.

S. officinalis × *S. tomentosa* and *S. officinalis* × *S. ringens*, under normal irrigation, had the largest above ground/root dry weight ratio, which under water stress remained unchanged in the former, while in *S. officinalis* × *S. ringens* was significantly increased, indicating a possible higher long-term sensitivity of this hybrid to water stress. Both *S. ringens* hybrids presented a significant reduction of root biomass under restricted irrigation, although their above ground biomass was not reduced. When the substrate contained attapulgite, although *S. ringens* hybrids still had their root biomass reduced under water restriction, the above ground/root dry weight ratio did not change, due to simultaneous reduction in shoot elongation, possibly providing better equilibrium of plants in case of limited water supply. Enhanced root growth in plants is fundamental to improve substrate water exploration and drought tolerance and there are numerous reports about the importance to enlarge root-to-shoot ratios in order to obtain drought tolerant genotypes and improve yield under water stress in various crops [43–45].

Attapulgite was also beneficial for *S. officinalis* × *S. tomentosa*, as in its presence the plant growth related parameters were not reduced when sparse irrigation was applied, and the above ground/root fresh and dry weight ratios were reduced, indicating a stronger root system capable of supporting the plant under water restriction. Although research on the effect of attapulgite on growth of various crops is limited, in sweet potato plants the application of attapulgite in the soil has been reported to enhance drought tolerance [33]. Possibly a lower than the 25.0 g/L concentration of attapulgite could be more beneficial to hybrids growth, based on the results of the experiment with *S. fruticosa*, which showed that attapulgite at 6.25 or 12.5 g/L increased above-ground fresh and dry weight compared to the 25.0 g/L and the control, although also in *S. fruticosa* the 25.0 g/L promoted root growth under limited irrigation. Attapulgite had a beneficial effect on retaining moisture in the substrate and therefore could be added to the substrate in xeriscaping and extensive green roofs enhancing the plants' drought resistance.

Most hybrids flowered at rather low percentage (40–80%) and intense (one inflorescence per plant), whereas plants of *S. fruticosa* and *S. officinalis* × *S. pomifera* did not flower at all. This is probably because cuttings were collected from mother plants that were only 8 months old and thus might have not reach full maturity. Alternatively, it is possible that stems should reach a certain length before they flower. In case of *S. fruticosa*, although mother plants were almost 3 years old, they had never been left to flower because of continuous collection of cuttings every 3 months. Although delayed flowering is an undesirable trait for ornamental crops, if improved *Salvia* cultivars were asexually propagated directly from adult hybrid populations, this could lead to faster flowering of the plants produced.

5. Conclusions

Crossability among the five selected *Salvia* species native to Greece and the eastern Mediterranean was low in most crosses; however, a number of interspecific hybrids with desirable ornamental traits and increased drought resistance were developed. *S. officinalis* × *S. ringens* and *S. officinalis* × *S. tomentosa* developed a compact plant shape and most lateral shoots, although pruning to enhance lateral shoot sprouting was not applied. All hybrids survived water stress better than *S. fruticosa*, especially *S. officinalis* × *S. pomifera* and *S. officinalis* × *S. tomentosa*.

The addition of attapulgite in the substrate, under limited irrigation, induced in some hybrids reduction of the above ground/root biomass ratio and in *S. fruticosa* increased the root dry weight indicating increased drought resistance.

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