A first approach for evaluation, breeding and promotion of native to Greece sage species for use as landscape plants

M. Papafotiou[1,a], A.N. Martini[1], K.F. Bertsouklis[1], G. Vlachou[1], E. Kanellou[1], E.G. Stylias[2], A. Kalantzis[2], L. Tassoula[1] and E. Dariotis[2]

[1]Laboratory of Floriculture and Landscape Architecture, Department of Crop Science, School of Plant Sciences, Agricultural University of Athens, Iera Odos 75, 118 55 Athens, Greece; [2]Kalantzis Plants, Agioi Saranta, 190 07 Marathon, Greece.

Abstract

Following the current tendency for xeriscaping and considering the ecological, environmental, economic and aesthetic advantages of native plants, clones of native in Greece sage species were evaluated and interspecific crossings were performed. Aim was to obtain hybrids with floricultural characteristics sought on the international market of ornamental herbs, such as compact plant shape, abundant and prolonged flowering, drought resistance, and ease of growth. *Salvia fruticosa* and *S. officinalis* were crossed with *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa* and each clone was used both as a pollen and seed parent for all crossings. Crossability was observed only when *S. officinalis* and *S. fruticosa* were used as seed parent, apart from the cross *S. tomentosa* × *S. fruticosa* that succeeded with *S. fruticosa* as pollen parent too. Hybrids with desirable characteristics were selected for further experimentation concerning substrate and fertilization and drought tolerance in a green roof. *S. officinalis* × *S. ringens* and *S. officinalis* × *S. tomentosa* developed a compact plant shape and the most lateral shoots. All hybrids survived water stress better than *S. fruticosa*, especially those of *S. ringens*. Germination of all species was highest at 10-15°C, indifferent to photoperiod, however *S. tomentosa* and *S. ringens* in all germination trials showed very low germination ability (less than 20%) and the other three species had an unstable response. *S. pomifera* ssp. *pomifera* presented up to 45% germination in peat-perlite without any pre-treatment, while *S. fruticosa* and *S. officinalis* up to 85% in MS medium, after scarification. Propagation by cuttings was highly successful (70-96%, average of four seasons) for all species and hybrids. As regards micropropagation, shoot multiplication was favoured in MS medium with 0.4 mg L^{-1} BA and 0.01 mg L^{-1} NAA, and rooting in half-strength MS medium with 0.25-1.0 mg L^{-1} IBA, while plantlets acclimatized ex vitro at percentages higher than 80%. All species, except *S. fruticosa,* and four selected hybrids, *S. fruticose* × *S. ringens*, *S. officinalis* × *S. ringens*, *S. officinalis* × *S. pomifera* ssp. *pomifera, S. officinalis* × *S. tomentosa*, survived drought stress in an extensive green roof and proved suitable for xeriscaping.

Keywords: *Salvia fruticosa*, *Salvia officinalis*, *Salvia pomifera*, *Salvia ringens*, *Salvia tomentosa*, native Mediterranean ornamentals, xeriscaping

INTRODUCTION

Salvia species, part of the macchia vegetation in eastern Mediterranean regions, are drought resistant, perennial, evergreen shrubs, which could be ideal for use as ornamental plants, in arid/semi-arid regions, particularly in xeriscaping. Native drought tolerant plants are ideal for xeriscaping, since, apart from having minimal irrigation requirements, usually present low cultivation needs and adaptability to different soil and climatic conditions, while they have high ornamental value, contribute to the preservation of the local landscape character, and promote bee-friendly landscaping, in support of combating pollinator

[a]E-mail: mpapaf@aua.gr

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population decline worldwide (Tassoula et al., 2021).

S. fruticosa Mill. (Greek sage; Figure 1a) is strongly aromatic, up to 120 cm tall with pink flowers in early spring. Endemic to the eastern Mediterranean, in Greece it is found in the central country, the Peloponnese, and the Aegean islands and has a long tradition of use, since the antiquity, as a medicinal, culinary, and melliferous plant. It is widely used for the preparation of herbal tea (faskomilo). *S. officinalis* L. (Dalmatian sage, common sage, sage; Figure 1b) is strongly aromatic, up to 60 cm tall, bearing violet-blue, pink or white flowers from May to July. It is naturally widespread on the Apennines and eastern Adriatic coast, though it has been naturalized in many places throughout the world (Tutin et al., 1972; Blamey and Grey-Wilson, 1993). In Greece it is found North and Eastern and in the Ionian islands. It is used since antiquity as a medicinal and culinary plant, being in nowadays one of the most important *Salvia* species worldwide, cultivated in many cultivars as medicinal and ornamental. *S. pomifera* L. is an endemic species of the eastern Mediterranean (Blamey and Grey-Wilson, 1993). The subspecies *S. pomifera* ssp. *pomifera* (Figure 1c) is strongly aromatic, up to 100 cm tall that grows in Crete and the Peloponnese bearing pink-violet flowers in spring to early summer on elegantly curving inflorescences. *S. ringens* Sibth. & Sm. (Figure 1d) is a hardy herbaceous plant with a slightly woody base, up to 30 cm tall, with long (60 cm) branching flowering stems and violet-blue or blue flowers during late spring through summer. It is native in the southern and eastern parts of the Balkan Peninsula (Tutin et al., 1972) and in Greece, it is spreading North to the highlands of Macedonia and Epirus, Mount Olympus, and in Central Greece. It is resistant to low temperatures. *S. tomentosa* Mill. (balsamic sage; Figure 1e) is an up to 80 cm tall shrub, similar to *S. officinalis*, with light pink-violet flowers with reddish-brown calyces in late spring or early summer (Blamey and Grey-Wilson, 1993). It is native in southern Europe (mostly Balkan Peninsula and Crimea) and part of western Asia (Anatolia and Near East), in areas of macchia vegetation and on limestone slopes (Guner et al., 2000). It is found in northeastern and central Greece and the northeastern Aegean Islands (Dimopoulos et al., 2013).

Figure 1. Plants of *S. fruticosa* (a) *S. officinalis* (b) *S. pomifera* ssp. *pomifera* (c), *S. ringens* (d) and *S. tomentosa* (e) in the wild at blooming.

Hybrids between the above Greek *Salvia* spp. could benefit of the variety of flower colour, leaf shape and aroma of all species, early abundant flowering of *S. fruticosa*, low compact plant shape and cold tolerance of *S. ringens*, adaptability to wet and cold climate of *S. tomentosa*, drought and cold tolerance of *S. officinalis* and provide new horticulture products, either potted or landscape plants. Hybridization has been reported between *S. fruticosa* and *S. officinalis* (Dudai et al., 1999; Radosavljević et al., 2019) and for *S. tomentosa* with *S. officinalis* and *S. fruticosa* (Putievsky et al., 1990). Following breeding and new hybrids achievement, propagation and greenhouse cultivation protocols are necessary to be developed in order to introduce the new *Salvia* species into the horticultural industry. Studies on seed ecophysiology of various *Salvia* species reviled a wide range of temperatures from 10 to 30°C that may be optimal for germination depending on species (Gorai et al., 2011), also many reports refer seed pretreatment in order to succeed or increase germination (Khakpoor et al., 2015; Abdollahi et al., 2012). Vegetative propagation by cuttings is a fast, well-established method being simpler compared to other clonal methods (Nicola et al., 2005; Paradiković et al., 2013). Micropropagation as a clonal method for the rapid production of large quantities of quality plants has been proposed for *Salvia* species too; particularly the

most commercial ones (Sağlam et al., 2014; Petrova et al., 2015).

Therefore, a research program (SALVIA-BREED-GR (https://www.salvia-breed-gr.com/el/) was developed with the aim to obtain interspecific hybrids with floricultural traits sought in the international market of ornamental herbs, such as compact plant shape, abundant and prolonged flowering, drought tolerance and ease of growth. Propagation protocols by either seed, stem cuttings or tissue culture were studied, as well as greenhouse growth protocols concerning substrate type and fertilization, and drought tolerance in a green roof for the five species and the selected new hybrids.

MATERIALS AND METHODS

In order to achieve the target set to obtain new *Salvia* hybrids with floricultural traits sought in the international market of ornamentals, particularly for use in low water demanding landscapes, the following steps were taken:

- Evaluation, selection of individuals (clones) of the five species in their natural environment, and establishment in the greenhouse;
- Vegetative propagation of clones to obtain mother plants and then plants for crossbreeding experiments;
- Crossbreeding;
- Evaluation of hybrid growth in the greenhouse;
- Development of efficient cultivation protocols;
- Development of efficient propagation protocols;
- Growth and drought tolerance evaluation in a green roof;
- Registration of new hybrids (patents) and introduction to the market.

Breeding

Clones of *Salvia fruticosa*, *S. officinalis*, *S. pomifera* ssp. *pomifera*, *S. ringens,* and *S. tomentosa* were obtained from stem cuttings collected from wild individuals and grown in a greenhouse. *S. fruticosa* was collected from East Attika, *S. officinalis* and *S. ringens* from northern Greece, *S. pomifera* ssp. *pomifera* from the southeastern coast of Peloponnese and *S. tomentosa* from the North-Aegean Island of Thasos. One clone of each species was used for crossbreeding. Clones of *S. fruticosa* and *S. officinalis* were crossed with the clones of the other three species and each clone was used both as a pollen and seed parent for all crossings, following the methodology suggested by Tychonievich and Warner (2011) for other *Salvia* species. Pollinations were made from April through May. Crosses were rated successful if mature, viable seed was produced. All mature seeds were harvested 4-6 weeks after crossings and sown under intermittent mist to record germination.

Hybrid-growth and evaluation

All hybrids (seedlings) produced by crossbreeding were grown in the greenhouse, under 18°C minimum temperature. Seedlings were pinched and then terminal stem cuttings were excised regularly for propagation to obtain enough plants for evaluation. After flowering, hybrids with desirable characteristics (combined morphology of both parents, high vegetative propagation and growth rate, compact plant shape, flower colour, stability of morphology) were selected and further experimentation concerning substrate type and fertilization schedule for greenhouse growth, as well as drought resistance was carried out.

The substrates tested were either peat containing wood fibre (30% wood fibre) (PWF, Forest Gold, Pindstrup, Denmark, pH 5.0-5.5) or PWF:perlite (1:1, v/v). Two fertilization treatments were applied, i.e., a water-soluble fertilizer (10N-3P-11K) was applied either weekly or monthly. In early April, one-month old rooted cuttings of selected hybrids were potted on peat-perlite 2:1 (v/v) and treated with two irrigation schedules, i.e., either adequately (when substrate humidity 20-23%) or sparsely (8-13% substrate humidity). Plant growth was evaluated after three months of culture (Papafotiou et al., 2021).

Propagation

1. Seed germination.

Seeds of the five species were harvested from native populations in August and stored in the dark, at room temperature, for five months. Selected for viability seeds were put to germinate either in solid, half-strength MS medium or in peat-perlite 1:1 (v/v) or on filter paper moistened with water, at 10, 15, 20, and 25°C, either in the dark or under 16 h light/8 h dark photoperiod. Scarification pretreatment of seeds either with sandpaper (suitable for metal surfaces, for 1 min) or with dense H_2SO_4 (15 min immersion) was applied.

2. Stem cuttings.

Terminal stem cuttings, 10 cm long, were collected from two-year-old greenhouse-grown plants of the five *Salvia* species, in November 2020 and February, May, and August 2021, indicative of the four seasons. Cuttings were treated either with dusting powder Rhizopon (0.5% w/w IBA) or their bases were immersed for 1 min in solution IBA (50% ethanol) at 0 (control) or 500 or 1500 or 3000 or 4500 or 6000 mg L^{-1}. Cuttings of hybrids collected from one-year-old mother plants in November were treated with dusting powder Rhizopon. Then, cuttings were placed for rooting on peat-perlite 1:1 (v/v) in a mist for two weeks, followed by four weeks on the greenhouse bench in a semi-shaded location.

3. Micropropagation.

Shoot tip or nodal explants, were cultured for shoot multiplication on MS medium with 30 g L^{-1} sucrose either without plant growth regulators (control) or supplemented with 0.4, 0.8, 1.6 or 3.2 mg L^{-1} 6-benzyladenine (BA) and 0.01 mg L^{-1} 1-Naphthylacetic acid (NAA). Microshoots, 1.0-2.0 cm long, were cultured for in vitro rooting on half-strength MS medium with 20 g L^{-1} sucrose either without plant growth regulators (control) or with 0.25, 0.5, 1.0, 2.0, 4.0 mg L^{-1} indole-3-butyric acid (IBA). Rooted microshoots were transplanted ex vitro for acclimatization on peat-perlite 1:1 (v/v). All media were solidified with 8 g L^{-1} agar and their pH was adjusted to 5.7-5.8 before agar addition and autoclaving (121°C for 20 min). Cultures were maintained at 25°C and a 16-h photoperiod.

Drought tolerance evaluation in a green roof

Rooted cuttings of the five *Salvia* sp. and the selected hybrids were planted in April 2021 in containers with a green roof infrastructure fitted (moisture retention and protection of the insulation mat, drainage layer and filter sheet) and placed on a second-floor roof at the Agricultural University of Athens. The substrate (10 cm depth) used was grape marc compost: perlite:pumice (3:3:4, v/v). Two irrigation frequencies were applied, starting from every three days (adequate, 16-22% v/v substrate moisture content) and five days (sparse, 7-11% substrate moisture content) and followed (beginning of June) by every two and four days, respectively. After five months of cultivation, dry weight of foliage and root system was recorded.

Statistical analysis

Depending on the experiment, the significance of the results was tested by either 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, 2013, USA). Data regarding rooting percentages of cuttings were analysed after arcsine transformation.

RESULTS AND DISCUSSION

Breeding and hybrid selection

Crosses of *S. officinalis* with *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa* were successful only when *S. officinalis* was used as seed parent. Interspecific crossability was much lower (5-6%) compared to self-crossing of *S. officinalis* (80%). Similarly, crosses of *S. fruticosa* with *S. pomifera* ssp. *pomifera* and *S. ringens* succeeded only when *S. fruticosa* was used as

seed parent (8 and 19%, respectively). Crosses of *S. fruticosa* with *S. tomentosa* were successful both when *S. fruticosa* was used as seed and pollen parent and at quite higher rates (28 and 53%, respectively). Self-crossing of *S. fruticosa* was the most successful (92%). *S. fruticosa, S. officinalis* and *S. pomifera* have the same chromosome number (2*n*=14; Karousou et al., 2000), while *S. tomentosa* 2*n*=16 and *S. ringens* 2*n*=12 (Petrova et al., 2015), however, this was not a barrier for crossbreeding, as the genus *Salvia* is highly tolerant of aneuploidy in its hybrids (Tychonievich and Warner, 2011). Hybridization within these *Salvia* species has been previously recorded for *S. officinalis* with *S. fruticosa* (Dudai et al., 1999) and *S. tomentosa* with *S. officinalis* and *S. fruticosa* (Putievsky et al., 1990).

All hybrids produced were cultured in the greenhouse and evaluated for the desired characteristics (combined morphology of both parents, vegetative propagation and growth rate, compact plant shape, flower colour). Four of the hybrids stood out, namely one from each *S. fruticose* × *S. ringens*, *S. officinalis* × *S. ringens*, *S. officinalis* × *S. pomifera* ssp. *pomifera, S. officinalis* × *S. tomentosa* cross (Figure 2). All hybrids presented vigor growth without any effect of substrate type and fertilization frequency. *S. officinalis* × *S. ringens* and *S. officinalis* × *S. tomentosa* developed a compact plant shape and the most lateral shoots (Figure 2). All hybrids survived water stress better than *S. fruticosa*, especially *S. officinalis* × *S. pomifera* ssp. *pomifera* and *S. officinalis* × *S. tomentosa* (Papafotiou et al., 2021).

Figure 2. The hybrids *S. officinalis* × *S. tomentosa* (right), *S. officinalis* × *S. pomifera* ssp. *pomifera* (middle) and *S. officinalis* × *S. ringens* (left).

Propagation

1. Seed germination.

Seed viability was found to be very low in all five species of *Salvia*. Photoperiod was indifferent for germination except in some cases where long days were slightly promotive. In vitro, in MS medium, at 15°C, *S. fruticosa* and *S. officinalis* presented highest germination rates after scarification either with sandpaper or H_2SO_4 (64-85%) as shown for other *Salvia* too (Khakpoor et al., 2015). In peat-perlite and on filter paper, *S. fruticosa* showed highest germination (23-33%) at 10 and 15°C, without pretreatment compared to H_2SO_4 scarification, while *S. pomifera* ssp. *pomifera* presented the highest germination (38-45%) of all species also without pretreatment at 10, 15 and 20°C. Cardinal temperatures for germination were 5 and 25°C. This temperature range is consistent with previous reports on germination of Mediterranean *Salvia* sp. *S. ringens* and *S. tomentosa* in all germination trials showed very low germination ability (less than 20%).

2. Propagation by cuttings.

Stem cuttings of all species rooted at satisfactory percentages (>70%) in all seasons, depending on IBA treatment. Merged data of four seasons per IBA treatment are presented in Table 1. All treatments with IBA, either in the form of dusting powder or as a solution, were more effective in rooting cuttings than the control, confirming previous reports (Nicola et al., 2005), excepting in *S. tomentosa* and *S. ringens*, whose cuttings treated with the control rooted at percentages comparable to higher IBA concentrations. Besides, dusting powder and

solution at 1500 or 3000 mg L^{-1} proved the most effective treatments for all species (Table 1). Cuttings of the hybrids *S. officinalis* × *S. pomifera*, *S. officinalis* × *S. tomentosa*, *S. officinalis* × *S. ringens* and *S. fruticose* × *S. ringens*, collected in autumn and treated with dusting powder rhizopon, rooted at even higher percentages (87-96%, data not shown in Table) than the species.

Table 1. Mean rooting percentage (%) of terminal cuttings collected from greenhouse-mother plants of the five *Salvia* species during four seasons (merged data of four seasons), in marked treatments with IBA.

Salvia species	IBA powder (0.5% w/w)	IBA solution (mg L^{-1})						$F_{one\text{-}way}$
		0	500	1500	3000	4500	6000	
S. fruticosa	80.8 a	43.8 c	69.6 b	77.1 ab	80.8 a	77.1 ab	70.4 b	**
S. officinalis	63.1 a	36.9 c	55.6 ab	61.2 a	56.9 ab	65.2 a	70.6 a	*
S. pomifera Ssp. *pomifera*	77.1 a	44.2 b	57.9 ab	74.2 a	74.2 a	70.4 a	67.9 a	**
S. tomentosa	78.8 ab	62.9 d	75.4 abc	87.1 a	73.3 abc	61.3 d	45.0 d	**
S. ringens	73.3 a	37.5 d	52.9 b	53.3 ab	61.7 a	40.4 cd	50.0 b	**

Mean comparison per *Salvia* species with Student's *t*-test at $p \leq 0.05$; means followed by the same letter (a-d) were not significantly different at $p \leq 0.05$; * or **, significant at $p \leq 0.05$ or $p \leq 0.01$, respectively.
IBA powder: dusting powder Rhizopon; IBA solution (50% ethanol) immersion for 1 min.

3. Micropropagation.

Shoot multiplication of all five *Salvia* species presented rather low efficiency due to low number of shoots produced per explant (Table 2). Nodal explants, although in most treatments produced more shoots per explant compared to shoot tip explants, responded in lower percentages and formed higher number of hyperhydrated shoots. Therefore, the multiplication efficiency was similar for the two types of explants. Supplementing the MS medium with 0.4 mg L^{-1} BA and 0.01 mg L^{-1} NAA induced the best response in terms of multiplication in all species. Similar concentrations of BA and NAA were found efficient for *S. officinalis* and *S. fruticosa* micropropagation in previous reports (Arikat et al., 2004; Petrova et al., 2015). In vitro rooting took place in half-strength MS medium with low IBA concentrations and was 100% successful in *S. officinalis*, satisfying in *S. fruticosa* and *S. tomentosa*, average in *S. ringens*, while it has not been investigated yet in *S. pomifera* ssp *pomifera* (Table 2). Ex vitro acclimatization of the plantlets was highly successful (Table 2).

Drought tolerance evaluation in a green roof

Five months after planting in a green roof, *S. fruticosa* presented the lowest survival percentage (50%) under drought stress compared to all other species and hybrids (67-100%). Sparse irrigation reduced dry weight of both plant foliage and root system in all species and hybrids. Regarding species, the highest percentage of foliage-dry weight reduction was observed in *S. fruticosa* (48%) and the lowest in *S. officinalis* (28%) (Table 3). *S. fruticosa* although it grows naturally in southern areas compared to all other species apart from *S. pomifera*, it has been found to face surviving problems under drought stress (Papafotiou et al., 2021), to the contrary of *S. officinalis* that shows high drought resistance. As for the hybrids, those of *S. ringens*, i.e., *S. fruticose* × *S. ringens* and *S. officinalis* × *S. ringens,* presented the lowest reduction of foliage dry weight (25 and 32%, respectively). Besides, the reduction percentage of root dry weight was lowest in species *S. officinalis* and *S. ringens* (21 and 25%, respectively) and in hybrids *S. officinalis* × *S. pomifera* and *S. officinalis* × *S. ringens* (25 and 38%, respectively) (Table 3).

Table 2. The effectiveness of suggested protocols on micropropagation of *Salvia* species (concentrations of plant growth regulators in mg L^{-1}).

Salvia species	Shoot multiplication	Microshoot rooting	Ex vitro acclimatization
S. fruticosa	MS, 0.4 BA/0.01 NAA 67-80% shoot production, 1.6-2.0 shoots explant^{-1}, 0.7-0.8 cm shoot length	½ MS, 0.25 or 0.5 IBA 71% rooting, 2.6-2.9 roots shoot^{-1}, 1.8-1.9 cm root length	87%
S. officinalis	MS, 0.4 BA/0.01 NAA 63-77% shoot production, 2.5-2.6 shoots explant^{-1}, 2.5-3.7 cm shoot length	½ MS, 0.25 IBA 100% rooting, 3.9 roots shoot^{-1}, 1.4 cm root length	88%
S. pomifera ssp. *pomifera*	MS, 0.4 BA/0.01 NAA 46-73% shoot production, 1.0-1.2 shoots explant^{-1}, 1.2-2.6 cm shoot length	-	-
S. tomentosa	MS, 0.4 BA/0.01 NAA 48-64% shoot production, 1.0-1.6 shoots explant^{-1}, 4.2-4.6 cm shoot length	½ MS, 0.1-0.5 IBA 70-75% rooting, 3.2-3.4 roots shoot^{-1}, 1.7-1.9 cm root length	96%
S. ringens	MS, 0.4/0.01 NAA 30-87% shoot production, 1.2-2.6 shoots explant^{-1}, 1.5-2.6 cm shoot length	½ MS, 0.5 IBA 50% rooting, 3.4 roots shoot^{-1}, 1.2-1.6 cm root length	>80%

Table 3. Percentage of foliage and root system dry weight reduction, between normal and sparse irrigation, after cultivation of *Salvia* species and their hybrids on an urban Mediterranean green roof for five months.

Species and hybrids	Foliage dry weight reduction (%)	Root dry weight reduction (%)
S. fruticosa	48	70
S. officinalis	28	21
S. pomifera ssp. *pomifera*	37	48
S. ringens	35	25
S. tomentosa	35	59
S. officinalis × *S. pomifera*	41	25
S. officinalis × *S. ringens*	25	38
S. fruticose × *S. ringens*	32	52
S. officinalis × *S. tomentosa*	38	48

CONCLUSIONS

Crossability among the five selected *Salvia* species native to Greece was low in most crosses, however a number of interspecific hybrids with desirable ornamental traits and drought resistance were developed. Propagation by cuttings was effective all year round and greenhouse cultivation was easy. The hybrids *S. officinalis* × *S. ringens* and *S. officinalis* × *S. tomentosa* developed an attractive compact plant shape and the most lateral shoots, while all selected hybrids survived drought stress better than *S. fruticosa* in a green roof.

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LUH 2021
VIII International Conference on

AGRICULTURAL UNIVERSITY OF ATHENS
Laboratory of Floriculture and Landscape Architecture

A first approach for evaluation, breeding and promotion of native to Greece sage species for use as landscape plants

Papafotiou M.,
Martini A.N., Bertsouklis K.F, Vlachou G., Kanellou E., Stylias, E.G, Kalantzis, A., Tassoula, L., Dariotis, E.

PROGRAMME:
Salvia-Breed-GR
www.salvia-breed-gr.com
mpapaf@aua.gr

European Union
European Regional Development Fund
HELLENIC REPUBLIC
MINISTRY OF ECONOMY & DEVELOPMENT
SPECIAL SECRETARY FOR ERDF & CF
MANAGING AUTHORITY OF EPAnEK
EPAnEK 2014-2020
OPERATIONAL PROGRAMME
COMPETITIVENESS
ENTREPRENEURSHIP
INNOVATION
ΕΣΠΑ 2014-2020
Partnership Agreement 2014 - 2020
Co-financed by Greece and the European Union

Following the current tendency for xeriscaping
and considering the ecological, environmental, economical and aesthetic advantages of native plants,
clones of native in Greece sage species were evaluated and interspecific crossings were performed

Salvia species studied in the research program

S. fruticosa *S. officinalis* *S. pomifera* *S. ringens* *S. tomentosa*

Aim was to obtain *Salvia* hybrids with floricultural characteristics
sought on the international market of ornamental herbs, such as
compact plant shape,
abundant and prolonged flowering,
drought tolerance and
ease of growth

***Salvia fruticosa* (Greek sage)**

strongly aromatic perennial

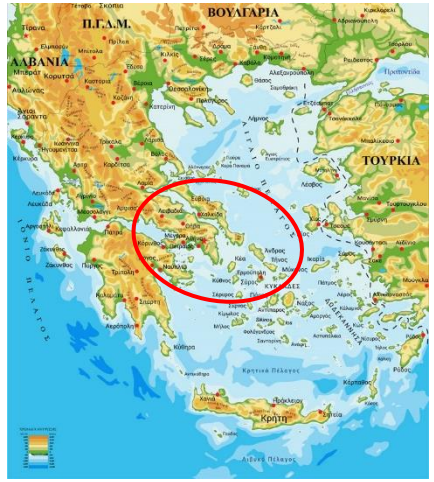
up to 1.20 m high

flowers lilac, pink (1.6-2.5 cm long) in early spring

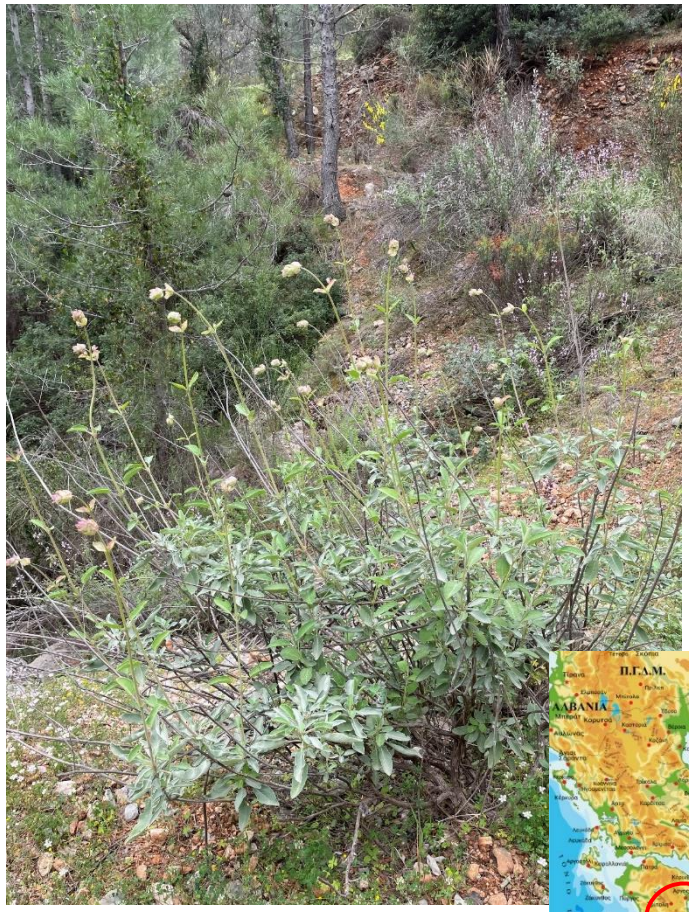
found in bushy rocky areas, often on coastal cliffs

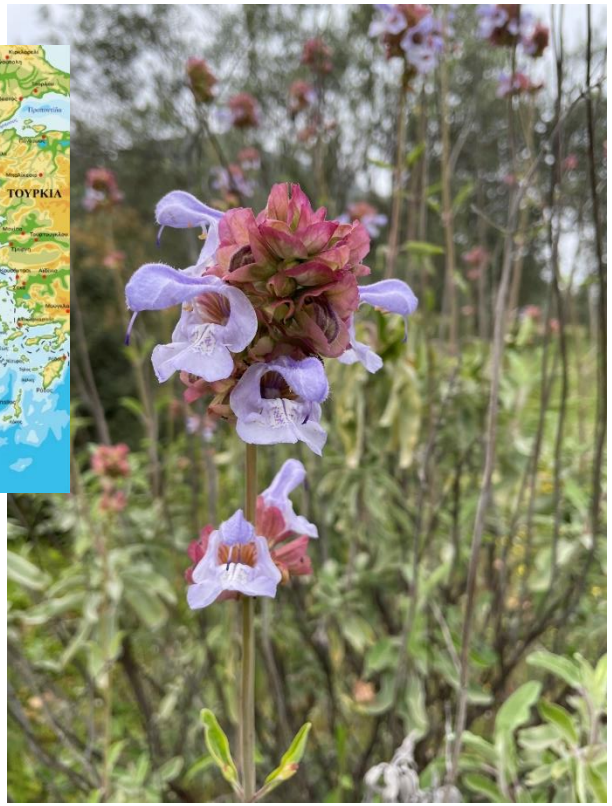
endemic to the Mediterranean zone from Sicily to Israel

in Greece, it is found in Central Greece, the Peloponnese and the Aegean islands



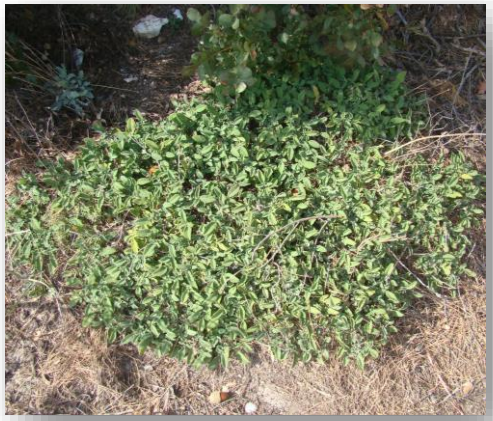
***Salvia officinalis* (sage)**
strongly aromatic perennial
up to 0.60 m high
flowers violet-blue, pink or white (2.0-3.5 cm long) in May – July
found in garrigue, rocky pastures, scrub, rocky places
naturally widespread on the Apennines and eastern Adriatic coast,
naturalized in many places throughout the world
cultivated in many varieties as medicinal and ornamental
in Greece it is found North and Eastern and in the Ionian islands





Salvia pomifera* ssp. *pomifera
strongly aromatic perennial
up to 1.00 m high
flowers pink and violet in spring to early summer on elegantly curving inflorescences
calyx often reddish-purple
found in dry, sunny places with phrygian vegetation and on rocky hillsides
endemic in Crete and the Peloponnese

Salvia ringens
hardy herbaceous perennial
up to 0.30 m high
flowering stems tall (60 cm), branching
flowers violet-blue or blue, large (about 3.8 cm long), late spring through summer
in areas with macchia vegetation, forest glades and streams
found in South and Eastern parts of Balkan Peninsula
in Greece, north to the highlands of Macedonia and Epirus, in Mount Olympus (up to 1,900 m)
resistant to low temperatures

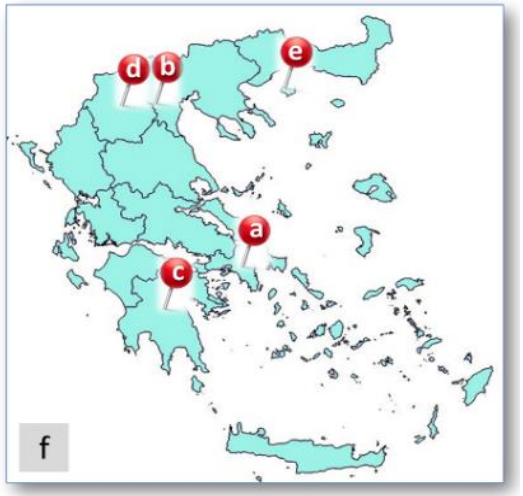


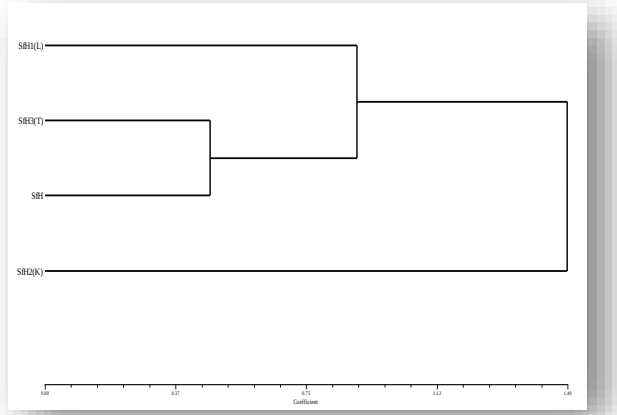
***Salvia tomentosa* (*S. grandiflora*, balsamic sage)**
perennial semi-woody herbaceous
up to 0.80 m high
flowers usually violet with reddish-brown calyces in late spring or early summer
found in macchia vegetation and on limestone slopes
in Southern Europe (mostly Balkan Peninsula and Crimea) and part of Western Asia (Anatolia and Near East)
in Greece, it spreads in the North-Eastern and Central country and the North-Eastern and Eastern Aegean Islands

The steps of the work:

- Evaluation and selection of clones of the 5 species
- Breeding
- Propagation
- Hybrid growth evaluation
- Green roof growth/drought tolerance evaluation

Evaluation and selection of clones of the 5 species

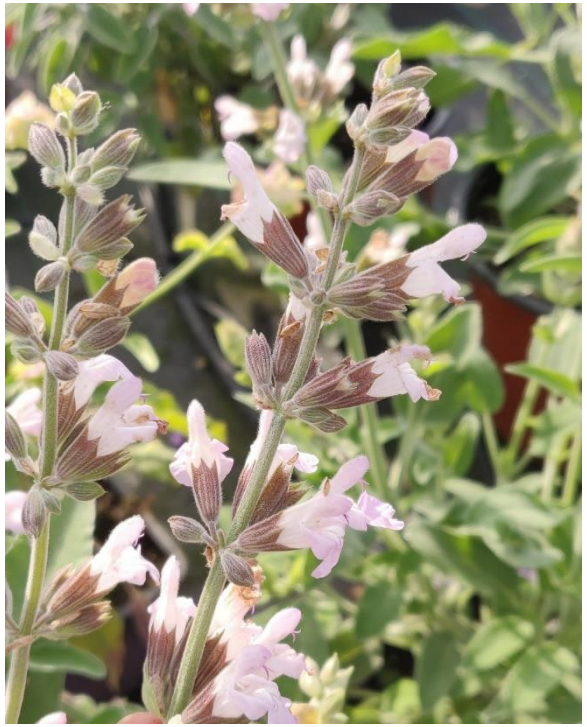




Clones of the five sage species were grown and evaluated in the greenhouse of the company KALANTZIS PLANTS, where breeding experiments were carried out

Breeding

Salvia fruticosa and S. officinalis were crossed with *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa* and each clone was used both as a pollen and seed parent for all crossings

crosses

X

Table 1. Interspecific crosses and self-pollinations of greek sage species (average data of 2019 and 2020 crosses).

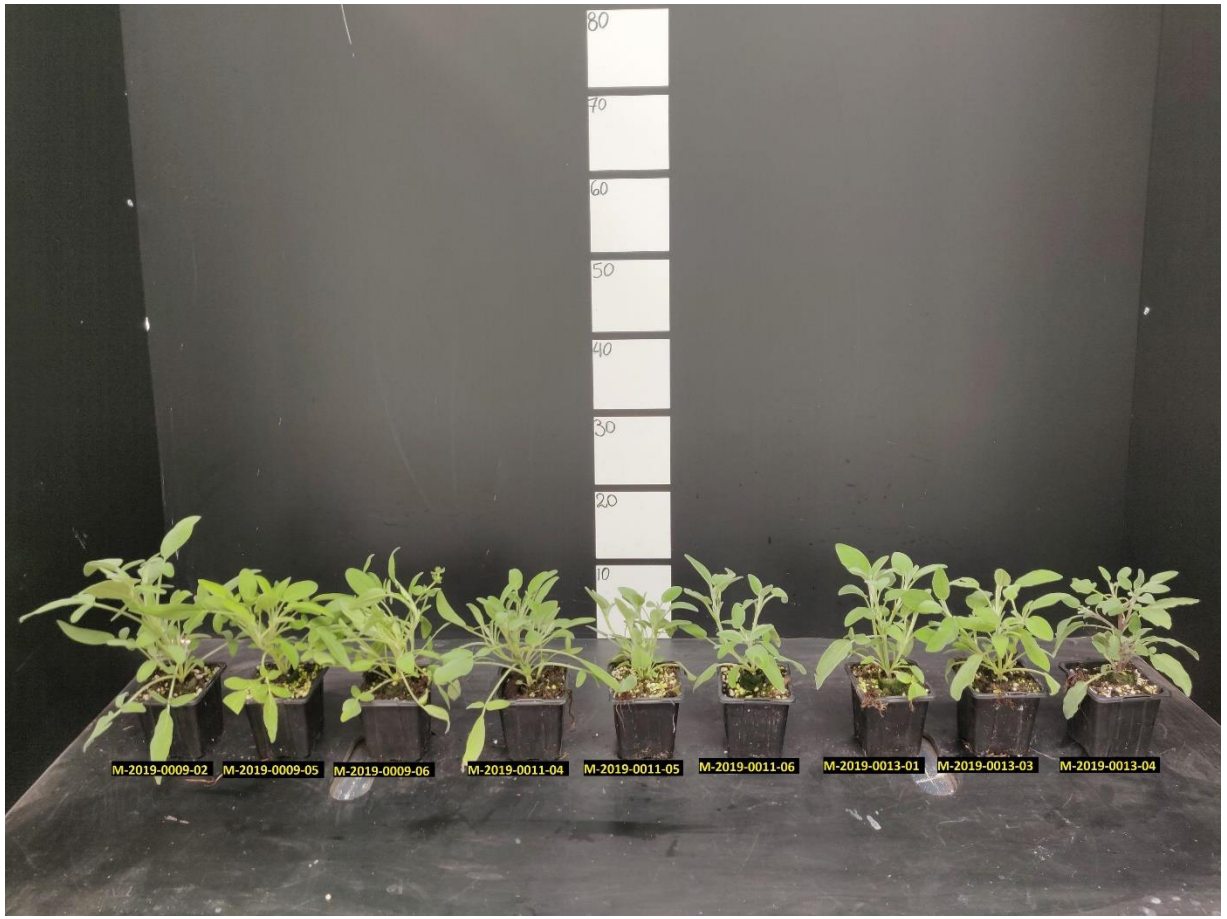
Cross	Pollinations number	Successful crosses (%)	Total seed number	Seedling number*
S. officinalis x self	50	80	40	29
S. officinalis x S. ringens	502	5.7	27	7
S. officinalis x S. pomifera	531	5.6	30	6
S. officinalis x S. tomentosa	389	6.4	24	5
S. ringens x S. officinalis	200	0	0	0
S. pomifera x S. officinalis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

Seedling number*: results of one year

Growth and selection of hybrids

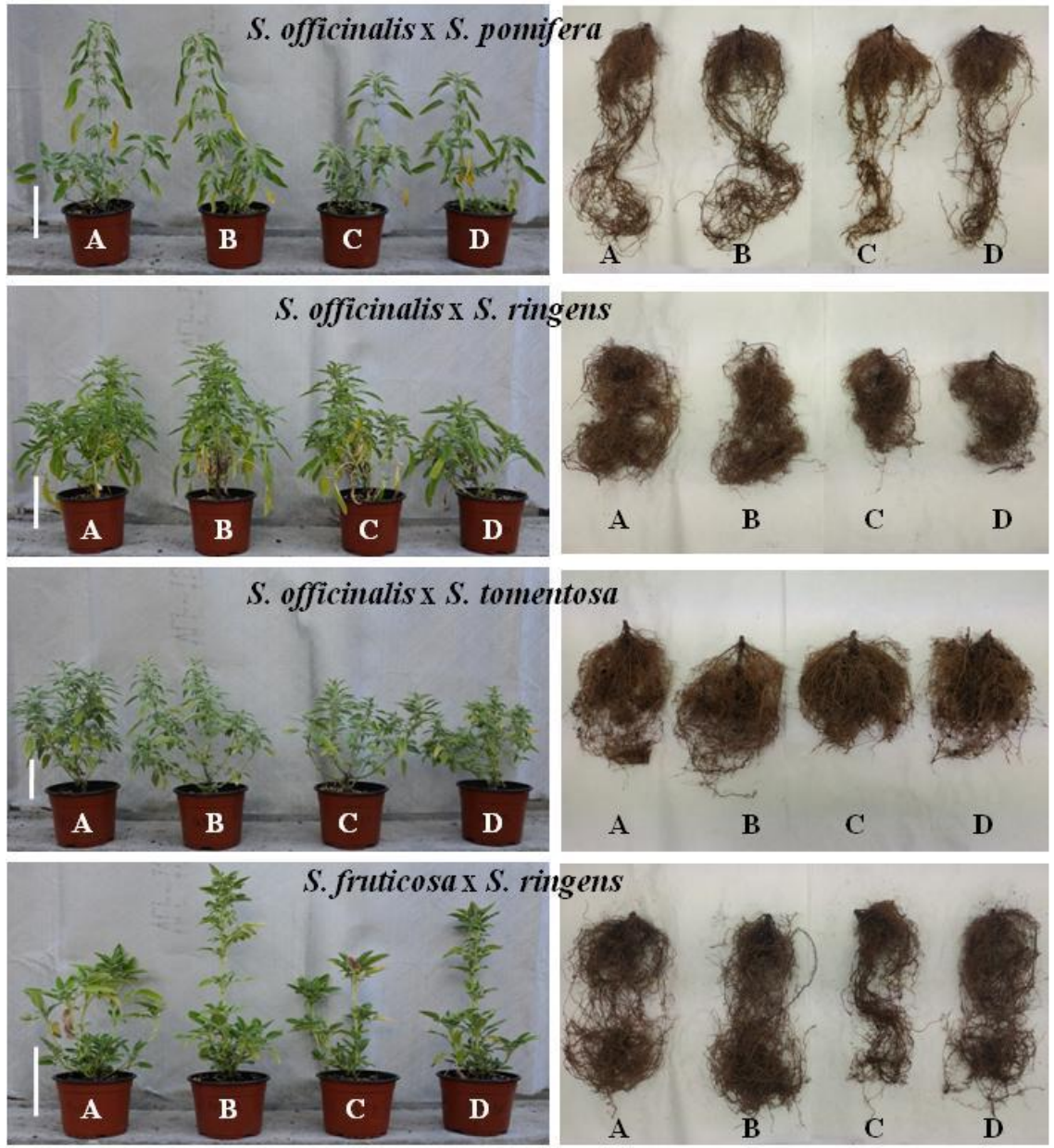
Final selection of 9 hybrids based on

- Ornamental characteristics
- Combination of both parental characteristics



Salvia hybrids

Evaluation of hybrids growth under different substrates and irrigation frequencies



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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.

Characteristics of the new hybrids

- Fast growth
- Easy production and propagation in commercial scale
- Ornamental value
- Drought tolerance

Propagation protocols

Stem cuttings

- The effect of season of collection
- The effect of plant origin (apical, basal)
- The effect of IBA concentration and method of application

Figure 1. Typical shoot-tip cuttings collected in spring of the species *Salvia fruticosa* (a), *S. officinalis* (b), *S. pomifera* ssp. *pomifera* (c), *S. ringens* (d) and *S. tomentosa* (e).

Propagation by cuttings was highly successful (70-100%) for all species and hybrids, excepting during summer when lower rooting rates were recorded (30-75%).

The most effective method for rooting stem cuttings of the 5 Salvia species was either treatment with

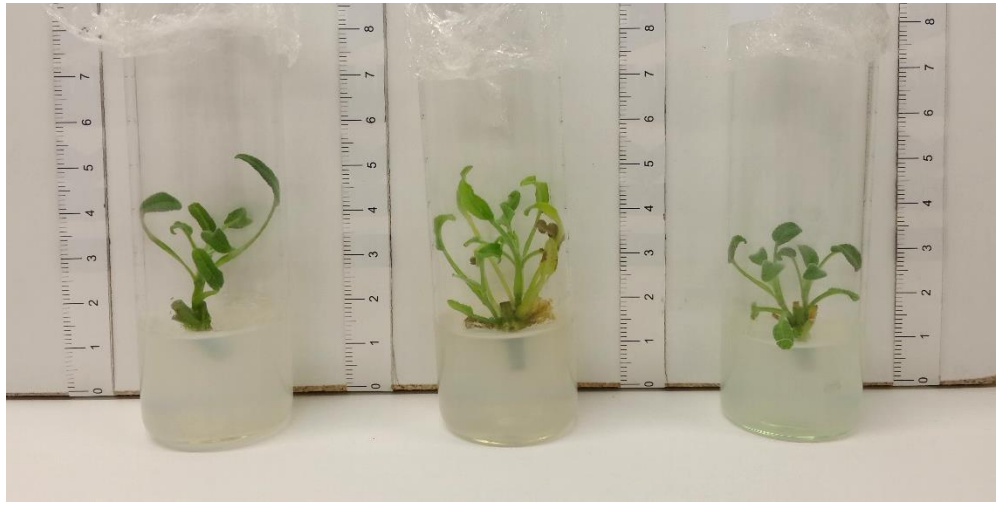
- dusting powder Rhizopon (0.5% w/w IBA) or
- immersion of the cutting base in a 2000 or 3000 mg /L IBA solution for 1 min.

TESTING PROPAGATION OF HYBRIDS IN COMMERCIAL SCALE

- Effect of treatments and season
- Evaluation of hybrids for propagation

In vitro propagation

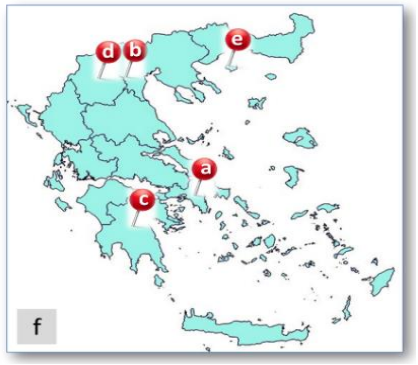
- Explant origin (mature plant, seedling/ shoot tip, axillary bud)
- Growth regulators type and concentration
- Rooting (IBA concentration)
- acclimatization



Shoot multiplication was favored on MS medium with 0.4 - 0.8 mg L^{-1} BA and 0.01 mg L^{-1} NAA

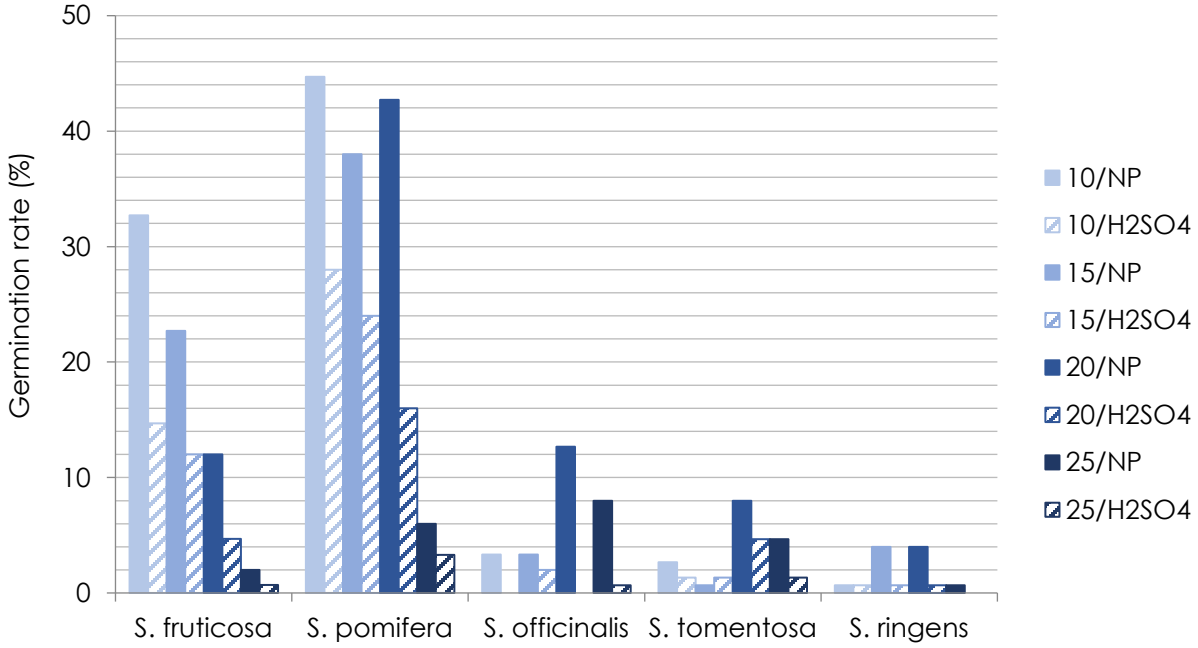
Microshoots rooted on half-strength MS medium with 0.25 - 1.0 mg L^{-1} IBA
Plantlets acclimatized *ex vitro* in peat-perlite (1: 1, v/v) at percentages higher than 80%

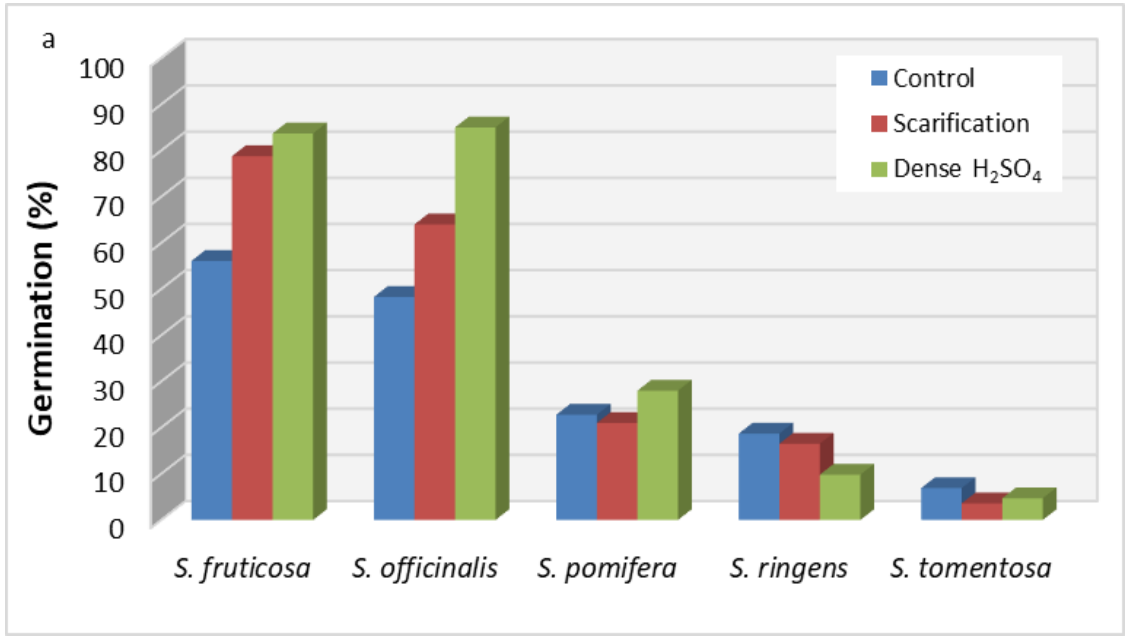
Seed propagation



Seed ecophysiology

- Germination temperature (best: 10 - 15 ºC)
- Photoperiod (light /dark)
- Seed pretreatment (GA3, scarification)





15 ºC/ 16 h Photoperiod

Species and hybrids evaluation for drought tolerance

Species and hybrids growth and drought tolerance evaluation in an urban extensive green roof

S. officinalis x S. ringens

S. officinalis x S. pomifera

S. officinalis x S. tomentosa

S. fruticosa x S. ringens

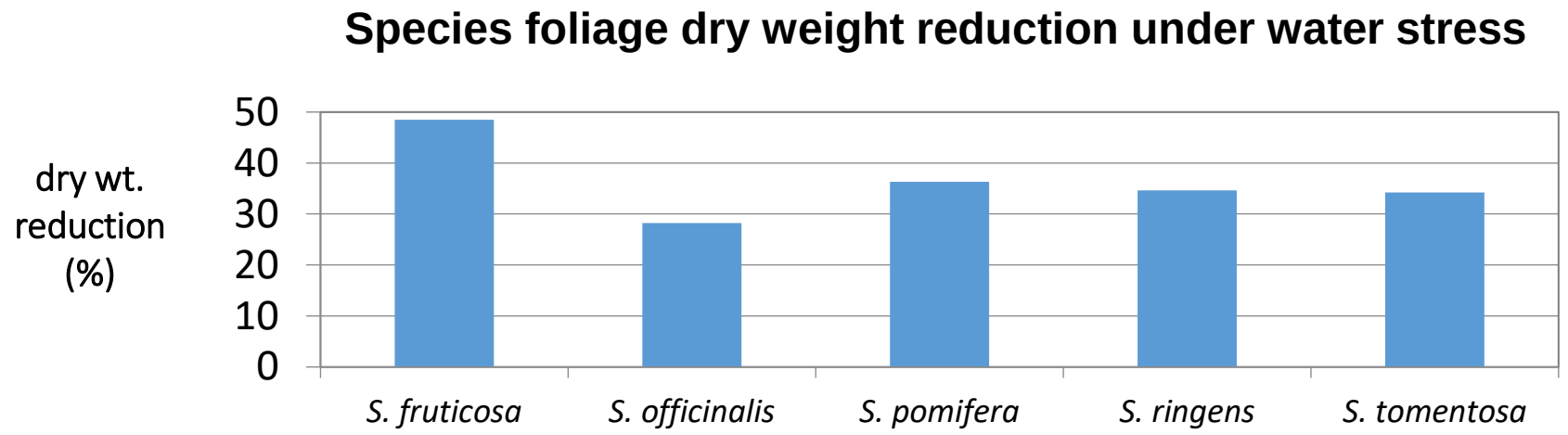
Drought tolerance evaluation of species and hybrids on a green roof

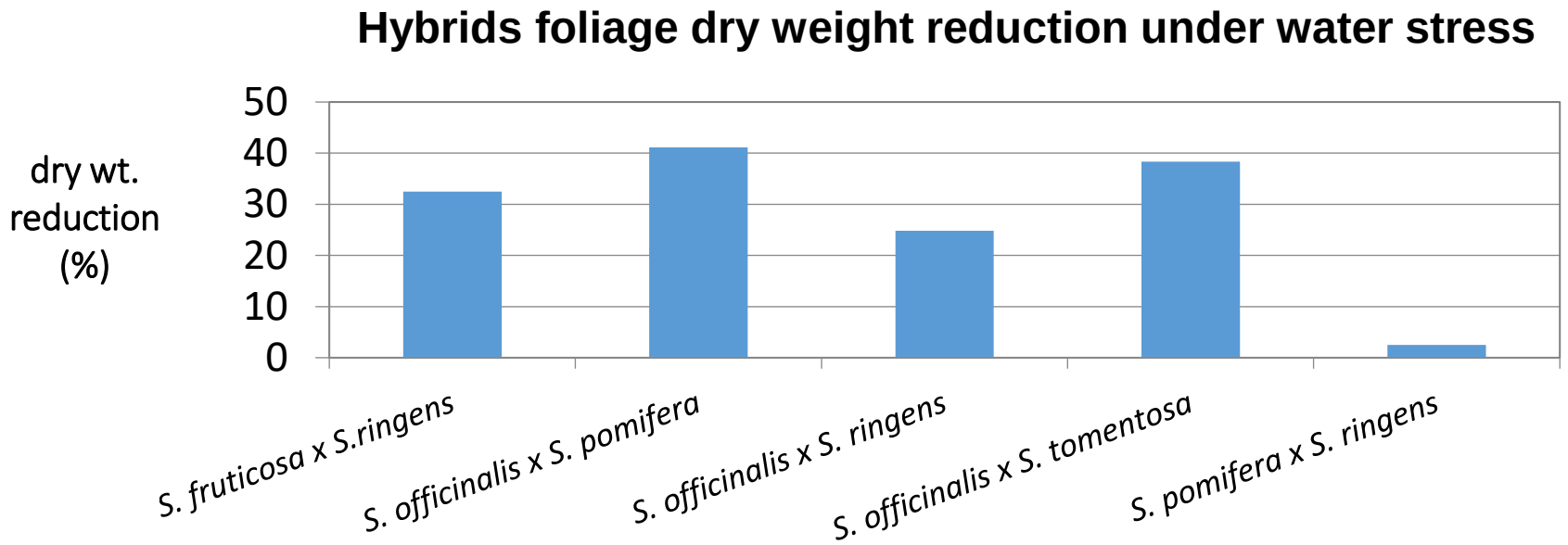
Growth parameters

Stomatal resistance

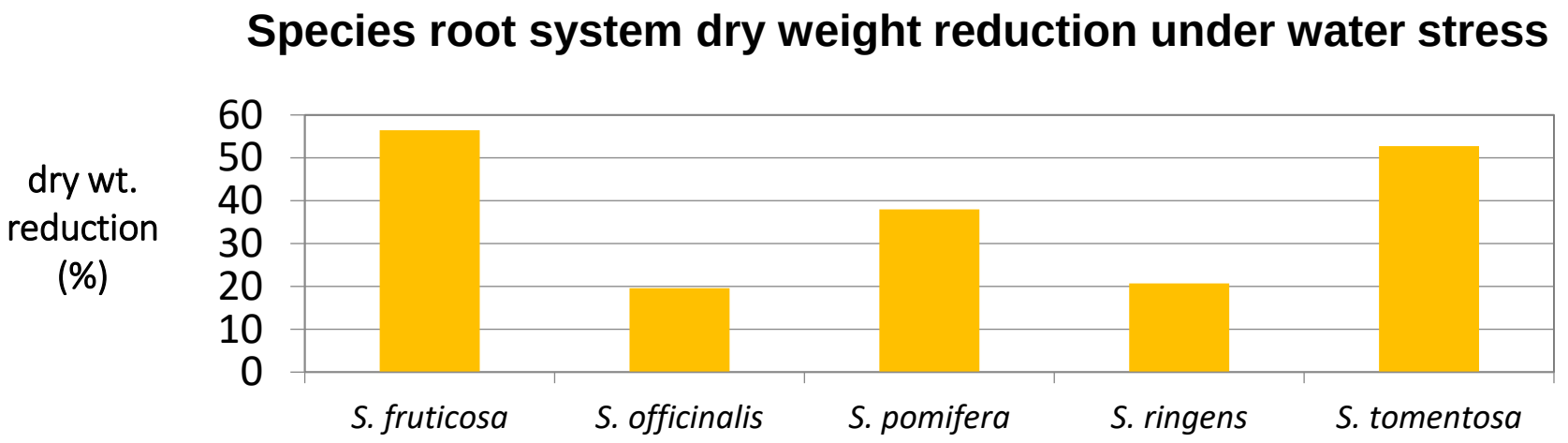
Maximum quantum yield of PSII (Φ_{PSIIo})

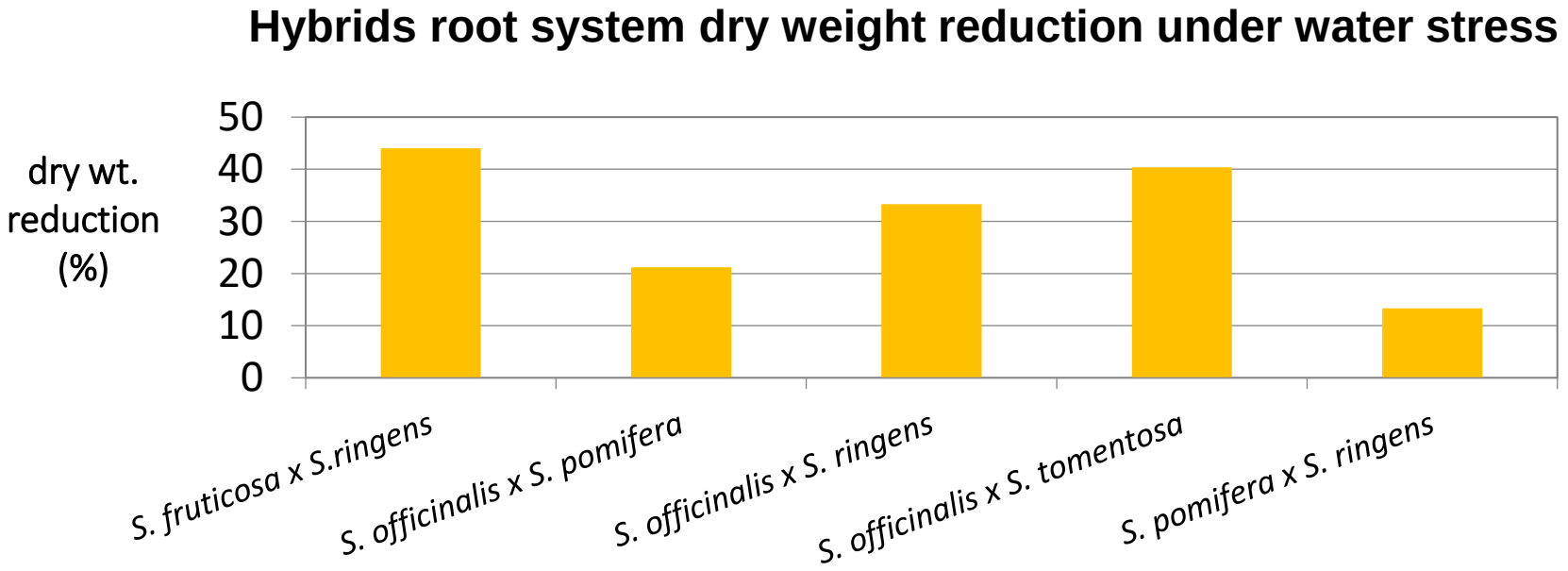
Drought tolerance evaluation of species and hybrids on a green roof





Drought tolerance evaluation of species and hybrids on a green roof

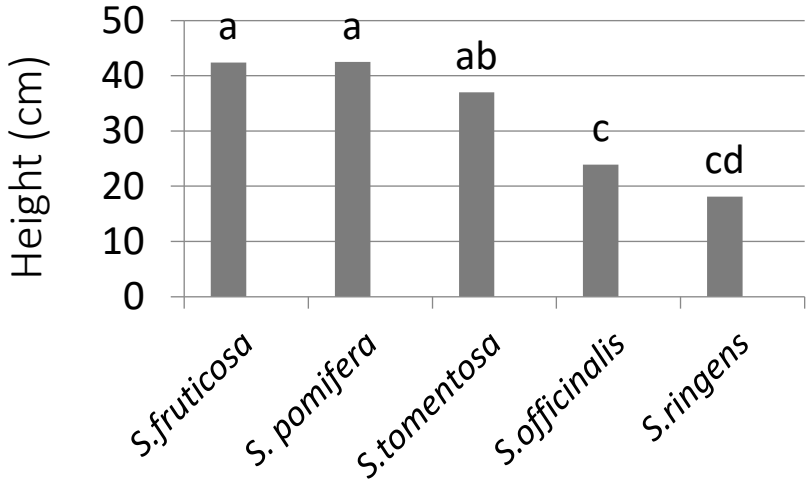


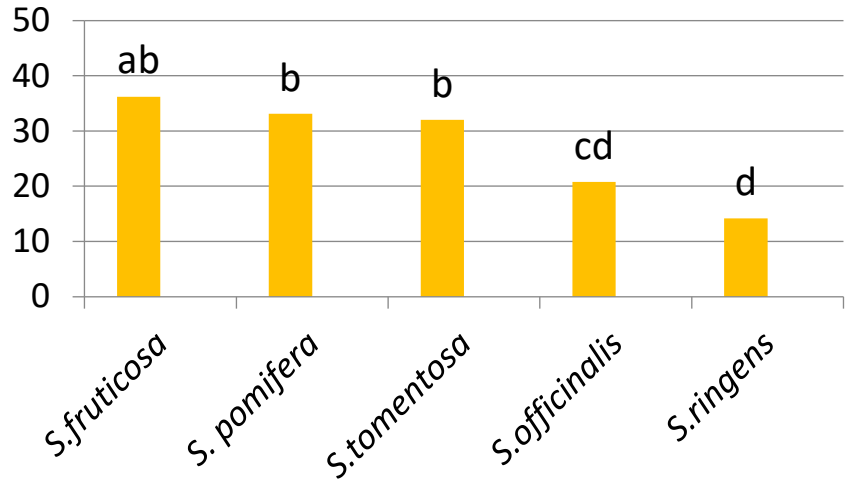


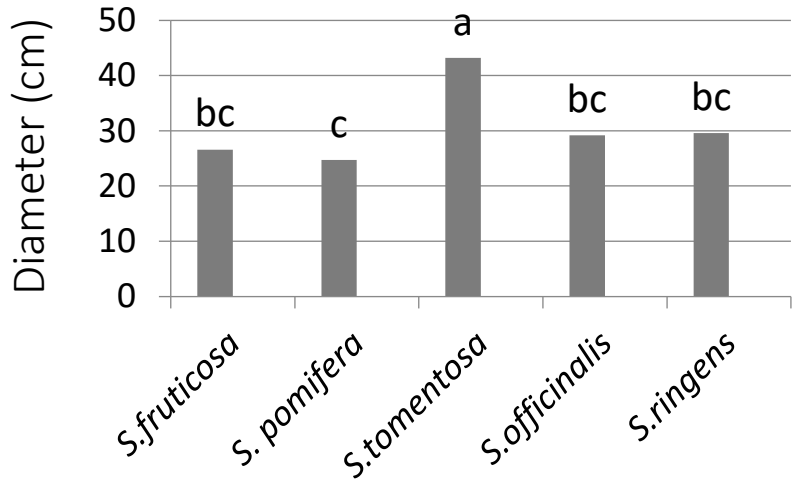
PARENTAL SPECIES' GROWTH (2020)

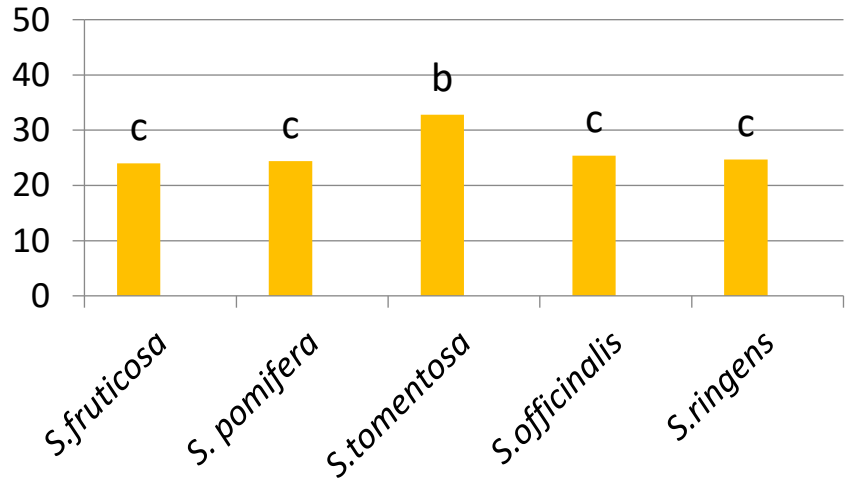
normal irrigation

sparse irrigation



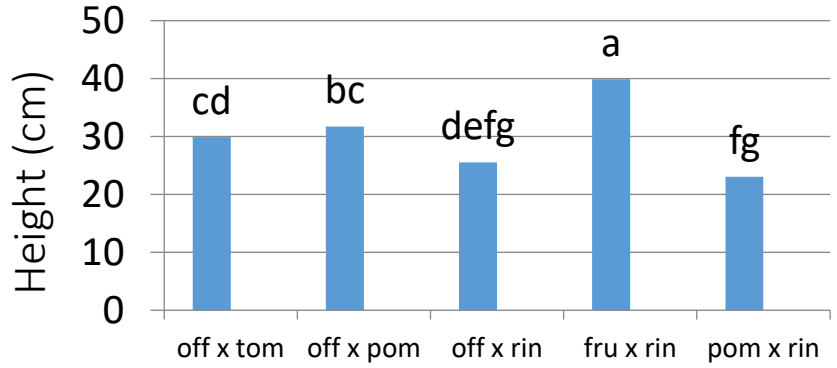


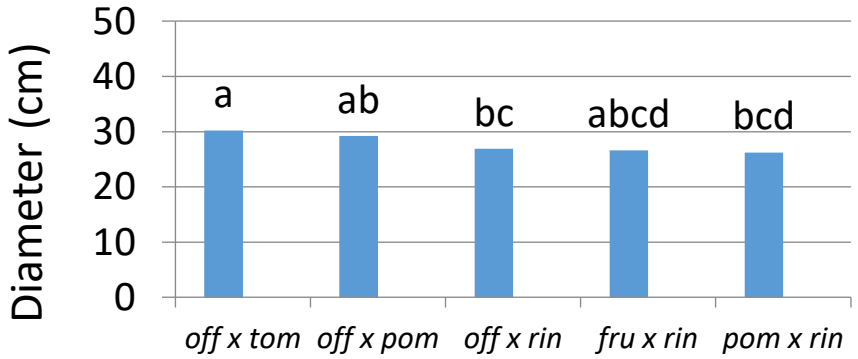




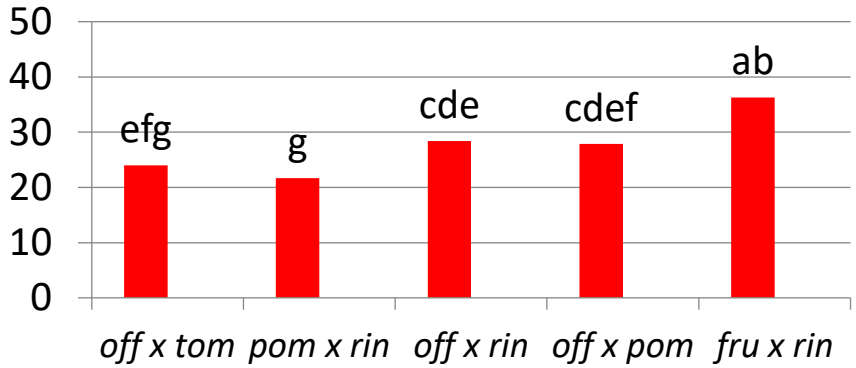
HYBRIDS' GROWTH (2021)

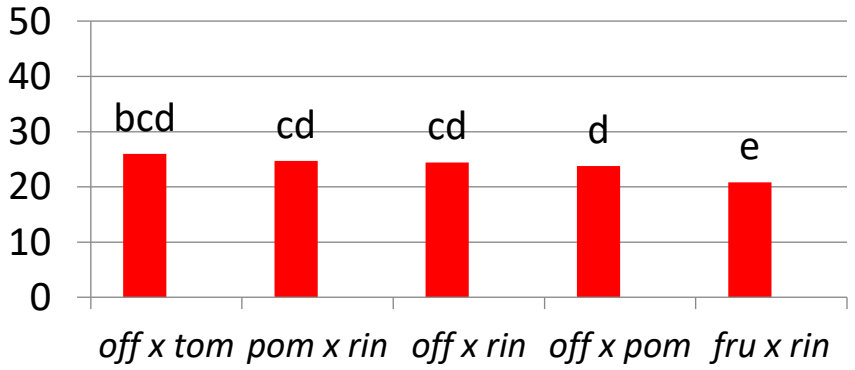
normal irrigation

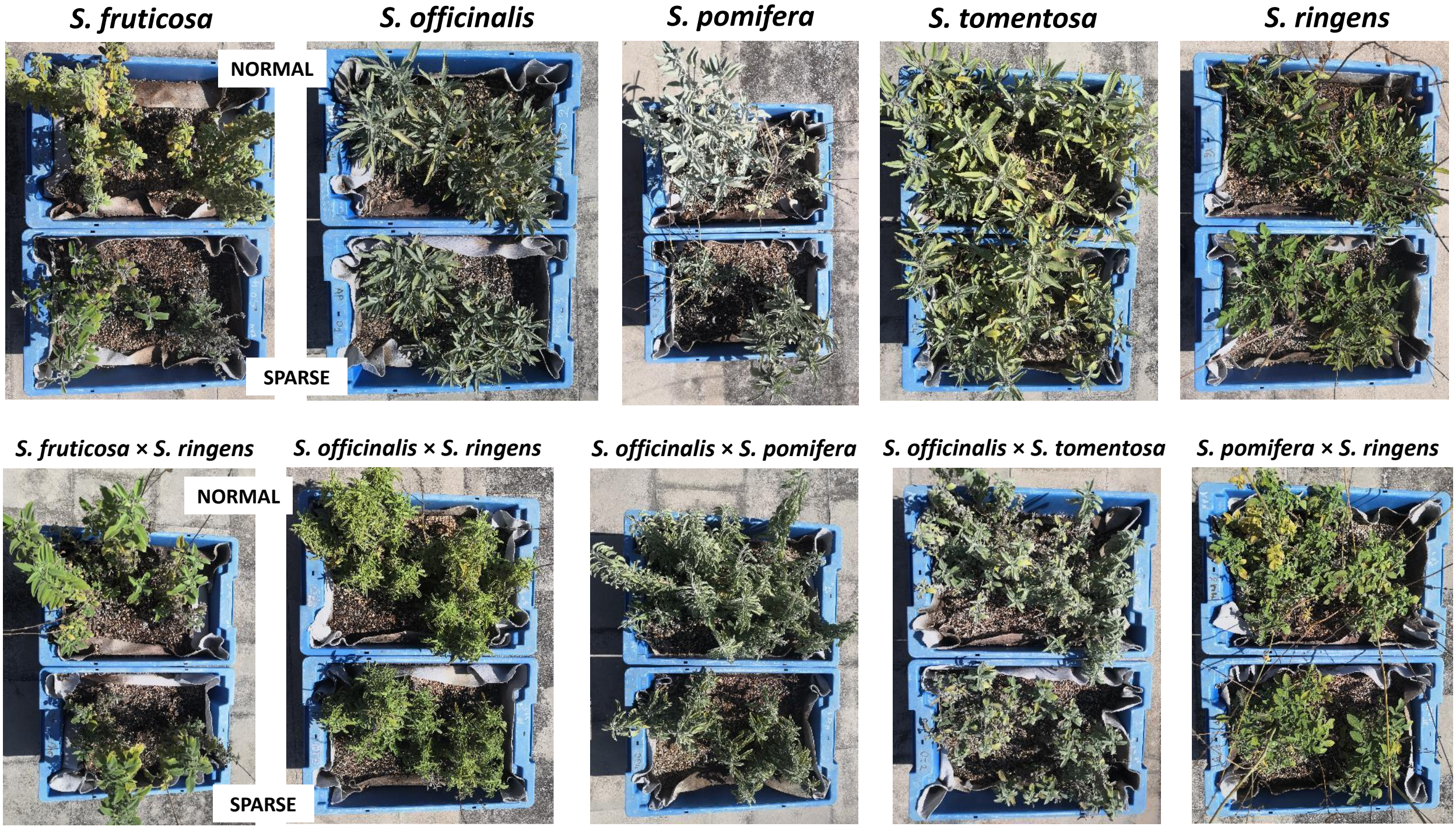




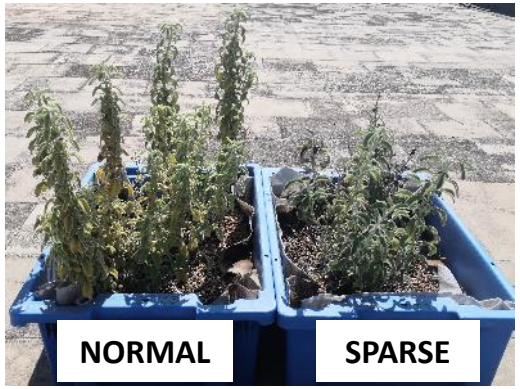
sparse irrigation






S. fruticosa
S. officinalis
S. pomifera
S. tomentosa
S. ringens
NORMAL
SPARSE
S. fruticosa × S. ringens
S. officinalis × S. ringens
S. officinalis × S. pomifera
S. officinalis × S. tomentosa
S. pomifera × S. ringens
NORMAL
SPARSE

S. fruticosa



S. officinalis

S. pomifera

S. tomentosa

S. ringens

S. fruticosa × *S. ringens*

S. officinalis × *S. ringens*

S. officinalis × *S. pomifera*

S. officinalis × *S. tomentosa*

S. pomifera × *S. ringens*

Conclusions

- Crossability among the five selected *Salvia* species native to Greece was low in most crosses, however a number of interspecific hybrids with desirable ornamental traits and drought resistance were developed
- The hybrids *S. officinalis* x *S. ringens, S. officinalis* x *S. tomentosa* and *S. pomifera* × *S. ringens* developed a compact plant shape and the most lateral shoots
- Hybrids survived water stress better than *S. fruticosa*, especially *S. pomifera* x *S. ringens*

S. officinalis* × *S. ringens

S. officinalis* × *S. tomentosa

S. officinalis* × *S. pomifera

S. pomifera* × *S. ringens

Thank you for your attention!

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