Interspecific crossability as a means to improve Mediterranean sage species with ornamental value

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

[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

In order to obtain new *Salvia* hybrids with desirable ornamental and commercial characteristics for the horticultural industry, interspecific crossings were done between five native to Greece *Salvia* species. The aim was to utilize particular characteristics, such as the bright flower colour of *S. pomifera* ssp. *pomifera*, the rich early flowering and drought tolerance of *S. fruticosa*, the low height of *S. ringens,* the adaptability to wet and cold climate of *S. tomentosa* and the cold and drought tolerance of *S. officinalis*. *S. officinalis* and *S. fruticosa* were not crossed with each other, because there have already been hybrids of them in the market. So, selected clones of these two species were crossed with selected clones of the other three species. Each clone was used both as a pollen and seed parent for all crossings. For each crossing at least 100 flowers were fertilized in order to estimate the effectiveness of fertilization. In spring 2020, *S. officinalis* was crossed with *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa*. Crossability was observed at much lower percentage (5-7%) in these three combinations compared to self-crossing of *S. officinalis* (80%), and only when *S. officinalis* was used as seed parent, whereas it was unsuccessful when used as pollen parent. *S. fruticosa,* which had very high self–crossability (92%), when used as seed parent, crosses with *S. ringens* and *S. tomentosa* were more successful (19 and 28%, respectively) than those with *S. pomifera* ssp. *pomifera* (8%). When *S. fruticosa* was used as pollen parent, only the cross *S. tomentosa* × *S. fruticosa* was feasible. Produced seeds were sown and hybrids with desirable characteristics were selected for further experimentation.

Keywords: *Salvia fruticosa*, *Salvia officinalis, Salvia pomifera*, *Salvia ringens, Salvia tomentosa*, native Mediterranean xerophytes, *Salvia* hybrids

INTRODUCTION

Interspecific hybridization is a common and important evolutionary mechanism in plants, which is used by plant breeders in order to create new plant forms or broaden genetic variability (Rodionov et al., 2019). Some of the economically important horticultural crops, such as the ornamentals *Rosa, Narcissus, Iris, Crocus* and *Chrysanthemum*, are of interspecific hybrid origin too (Van Tuyl et al., 2002).

Greek *Salvia* species, such as *S. fruticosa*, *S. officinalis*, *S. pomifera* ssp. *pomifera*, *S. tomentosa* and *S. ringens*, being drought resistant could be ideal plants for use as ornamentals, particularly in xeriscaping, as well as bee friendly and medicinal aromatic plants. Hybrids between *S. fruticosa* and *S. officinalis*, either artificial or natural, are known (Dudai et al., 1999; Radosavljević et al. 2019) and some of them are used commercially, while low crossability has been reported for *S. tomentosa* with *S. officinalis* and *S. fruticosa* (Putievsky et al., 1990).

Aim of the present study was, through the proposed crossings, to utilize particular characteristics of five selected *Salvia* species native to Greece, such as leaf shape, aroma, flower colour and inflorescence shape of all species, rich early flowering of *S. fruticosa*, low height of *S. ringens*, resistance to low temperatures of *S. officinalis*, *S. ringens* and *S. tomentosa*, as well as minimum water requirements of *S. fruticosa* and *S. officinalis*, in order to obtain

[a]E-mail: martini_agr@yahoo.com

hybrids with desirable ornamental and commercial characteristics and introduce new drought-resistant species in the floricultural industry. So, the possibility of interspecific crossbreeding among the five Greek sage species was studied and hybrids with desirable floricultural characteristics were selected for further experimentation.

MATERIALS AND METHODS

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 colour, time and duration of flowering, leaf aroma, as well as cold and drought resistance. 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 through March of year 2019-2020, where a minimum temperature of 18°C was maintained. From April through September, all plants were transferred to an insect-enclosed net greenhouse for the crosses. Plants began to bloom from March and flowered simultaneously up May.

Clones of *S. fruticosa* and *S. officinalis* were crossed with the clones of the other three species, *S. ringens*, *S. pomifera* ssp. *pomifera* and *S. tomentosa*, and each clone was used both as a pollen and seed parent for all crossings (Figure 1), following the methodology suggested by Tychonievich and Warner (2011) for other *Salvia* species. *S. officinalis* and *S. fruticosa* were not crossed with each other, because there have already been hybrids of them in the market.

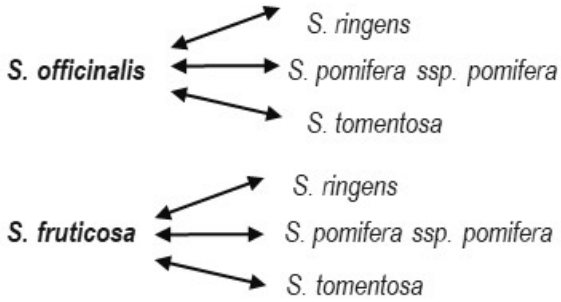


Figure 1. Schematic representation of interspecific crosses between *Salvia officinalis* or *S. fruticosa* and the other three *Salvia* species.

For each interspecific cross combination, at least 100 flowers were pollinated. Pollinations were conducted from April through May 2020, during the morning hours. 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. 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, although without emasculation. All mature seeds were harvested 4-6 weeks after crossings and stored in paper bags at room temperature for one month. Then, seeds were sown under intermittent mist with bottom heat to observe germination and seedlings were grown in the greenhouse. Hybrids were reproduced through stem cuttings and were maintained in the greenhouse in order to be evaluated for desirable characteristics.

RESULTS AND DISCUSSION

Interspecific crosses of *S. officinalis* with *S. ringens*, *S. pomifera* ssp. *pomifera*, and *S. tomentosa*, which were conducted in spring 2020, were successful at lower percentage (<7.5%), when *S. officinalis* was used as seed parent, compared to self-crossing of *S. officinalis* that was quite high (80%), whereas crossability was unsuccessful when *S. officinalis* was used as pollen parent (Table 1). Putievsky et al. (1990) also reported extremely low crossability

between *S. officinalis* and *S. tomentosa* (2%), when *S. officinalis* acted as a pollen parent.

Table 1. Interspecific crosses and self-pollinations of *S. officinalis* (data of crosses 2020).

Cross	Pollinations number	Successful crosses (%)	Total seed number
S. officinalis × self	50	80	40
S. officinalis × *S. ringens*	138	5.8	8
S. officinalis × *S. pomifera*	111	5.4	6
S. officinalis × *S. tomentosa*	165	7.3	12
S. ringens × *S. officinalis*	100	0	0
S. pomifera × *S. officinalis*	100	0	0
S. tomentosa × *S. officinalis*	100	0	0

Regarding interspecific crosses of *S. fruticosa* with the other three species, when *S. fruticosa* was used as seed parent, crosses with *S. ringens* and *S. tomentosa* were more successful than those with *S. pomifera* ssp. *pomifera*. When *S. fruticosa* was used as pollen parent, only the cross *S. tomentosa* × *S. fruticosa* was feasible and successful at quite high percentage (Table 2). Previous successful crossbreeding (21%) with *S. tomentosa* acting as seed parent and *S. fruticosa* as pollen parent has been reported (Putievsky et al., 1990). Self-crossing of *S. fruticosa* was the most successful (92%) (Table 2).

Table 2. Interspecific crosses and self-pollinations of *S. fruticosa* (data of crosses 2020).

Cross	Pollinations number	Successful crosses (%)	Total seed number
S. fruticose × self	50	92	48
S. fruticose × *S. ringens*	100	19	19
S. fruticose × *S. pomifera*	100	8	8
S. fruticose × *S. tomentosa*	100	28	28
S. ringens × *S. fruticosa*	100	0	0
S. pomifera × *S. fruticosa*	100	0	0
S. tomentosa × *S. fruticosa*	100	53	160

The five Mediterranean *Salvia* species native to Greece, belong to the *Salvia officinalis* group that consists of about ten perennial species, which are distributed in the Mediterranean Basin and the Near East (Putievsky et al., 1990). Although selected *Salvia* species have different chromosome numbers, i.e., *S. fruticosa*, *S. officinalis* and *S. pomifera* have 2*n*=14 (Karousou et al., 2000), *S. tomentosa* 2*n*=16 and *S. ringens* 2*n*=12 (Petrova and Vladimirov, 2020), this is not a barrier to the formation of hybrids, as the genus *Salvia* is considered highly tolerant of aneuploidy in its hybrids (Tychonievich and Warner, 2011). 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 (Putievsky et al., 1990).

Produced hybrids were evaluated in terms of their development (easy propagation by cuttings and cultivation inside a greenhouse, fast growth) and the desired characteristics, compact plant shape, leaf shape, colour and aroma, early and intense flowering,). Four of them stood out during the initial evaluation stages, due to their following basic characteristics:

- *S. officinalis* × *S. pomifera* (Figure 2a) produces a few long lateral shoots, seems to have intermediate characteristics of its parents in plant height, leaf colour and shape, and has a pleasant distinctive aroma, which is slightly closer to the aroma of *S. pomifera*. It has flowers which resemble those of *S. pomifera*, but they are born in shorter stems;
- *S. officinalis* × *S. ringens* (Figure 2b) is taller than *S. ringens* and produces many lateral shoots, while its segmented leaves are unique and particularly decorative, with very light aroma, like *S. ringens*. Flowering stems are about 40 cm long bearing more

flowers than *S. ringens*, light violet-blue in colour;

- *S. officinalis* × *S. tomentosa* (Figure 2c) is a compact plant with numerous lateral shoots and grey-green leaves smaller than those of *S. officinalis*. It also forms many flowering stems with light pink flowers and has a lighter aroma than *S. officinalis*;
- *S. fruticose* × *S. ringens* (Figure 2d) looks like *S. fruticosa* in height and shape, but it has intensely hairy segmented leaves, which have very light aroma like *S. ringens*. Its flowering stems are longer (about 80 cm) than those of *S. fruticosa*, while flowers are light purple and more sparsely arranged, characteristics inherited from *S. ringens*.

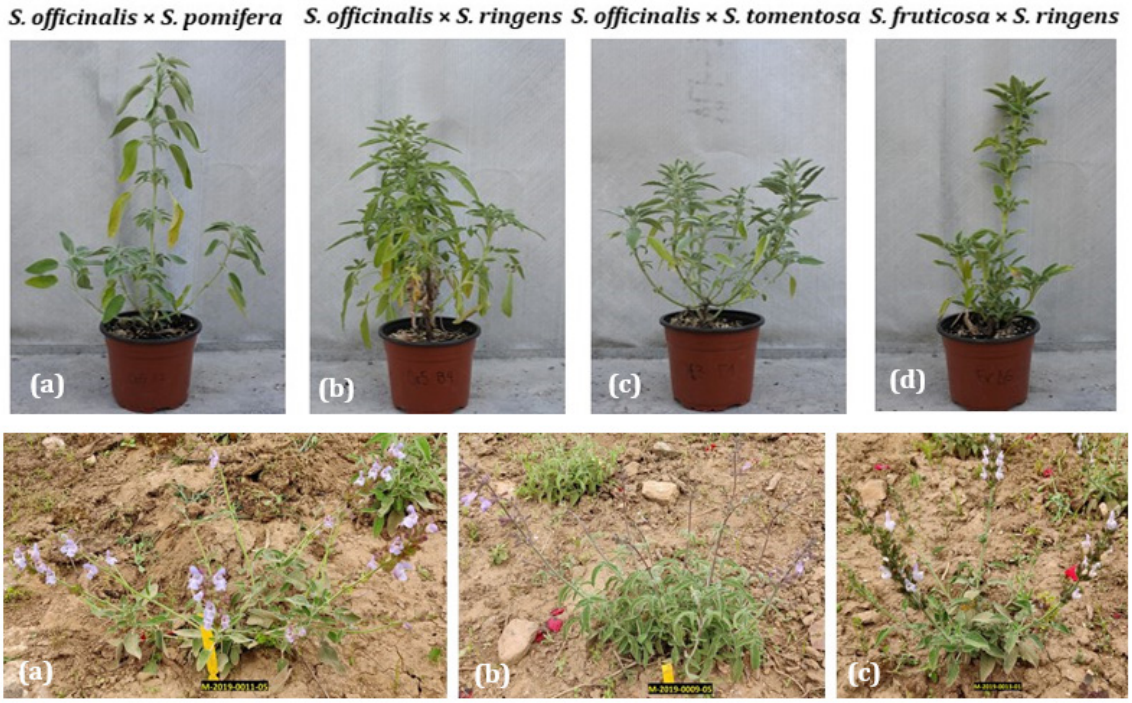


Figure 2. Selected sage hybrids with desirable characteristics, after three months of greenhouse cultivation under drought stress (up). Hybrids planted in the soil at the flowering stage in April (down).

Conclusively, crossability among the five selected *Salvia* species native to Greece and the eastern Mediterranean was low in most crosses and was feasible only when *S. fruticosa* or *S. officinalis* were used as seed parent, excepting the cross of *S. fruticosa* with *S. tomentosa*, in which *S. tomentosa* could be successfully used both as seed and pollen parent. Hybrids were reproduced by stem cuttings and maintained in a greenhouse, while a few hybrids with desirable ornamental characteristics were selected for further experimentation.

ACKNOWLEDGEMENTS

This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH - CREATE - INNOVATE (Project code: T1EDK-04923, Project: SALVIA-BREED-GR).

Literature cited

Dudai, N., Lewinsohn, E., Larkov, O., Katzir, I., Ravid, U., Chaimovitsh, D., Sa'ad, D., and Putievsky, E. (1999). Dynamics of yield components and essential oil production in a commercial hybrid sage (*Salvia officinalis* × *Salvia fruticosa* cv. Newe Ya'ar no. 4). J Agric Food Chem *47* (*10*), 4341–4345 https://doi.org/10.1021/jf9901587. PubMed

Karousou, R., Hanlidou, E., and Kokkini, S. (2000). The sage plants in Greece: distribution and infraspecific

variation. In Sage: The Genus *Salvia*, S.E. Kintzios, ed. (Amsterdam, The Netherlands: Harwood Academic Publishers), pp.32.

Petrova, A., and Vladimirov, V. (2020). Chromosome atlas of the Bulgarian vascular plants. Phytol. Balc. *26*, 217–427.

Putievsky, E., Ravid, U., Diwan-Rinzler, N., and Zohary, D. (1990). Genetic affinities and essential oil composition of *Salvia officinalis* L., *S. fruticosa* Mill., *S. tomentosa* and their hybrids. Flavour Fragrance J. *5* (*2*), 121–123 https://doi.org/10.1002/ffj.2730050213.

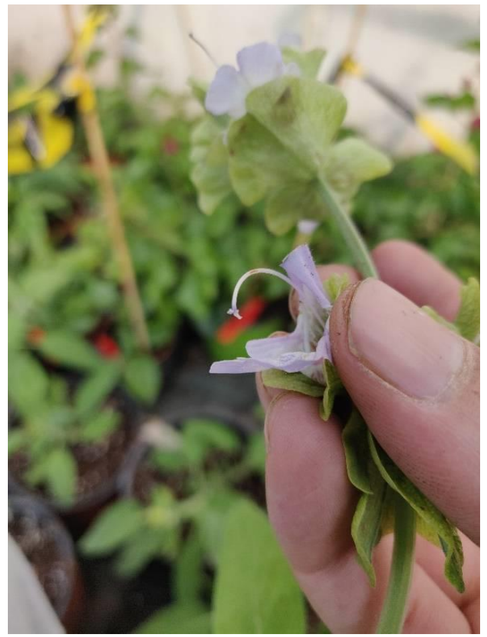
Radosavljević, I., Bogdanović, S., Celep, F., Filipović, M., Satovic, Z., Surina, B., and Liber, Z. (2019). Morphological, genetic and epigenetic aspects of homoploid hybridization between *Salvia officinalis* L. and *Salvia fruticosa* Mill. Sci Rep *9* (*1*), 3276 https://doi.org/10.1038/s41598-019-40080-0. PubMed

Rodionov, A.V., Amosova, A.V., Belyakov, E.A., Zhurbenko, P.M., Mikhailova, Yu.V., Punina, E.O., Shneyer, V.S., Loskutov, I.G., and Muravenko, O.V. (2019). Genetic consequences of interspecific hybridization, its role in speciation and phenotypic diversity of plants. Russ. J. Genet. *55* (*3*), 278–294 https://doi.org/10.1134/S1022795419030141.

Tychonievich, J., and Warner, R.M. (2011). Interspecific crossability of selected *Salvia* species and potential use for crop improvement. J. Am. Soc. Hortic. Sci. *136* (*1*), 41–47 https://doi.org/10.21273/JASHS.136.1.41.

Van Tuyl, J.M., Lim, K.B., and Ramanna, M.S. (2002). Interspecific hybridization and introgression. In Breeding for Ornamentals: Classical and Molecular Approaches, A. Vainstein, ed. (Dordrecht: Springer), pp.85 https://doi/org/10.1007/978-94-017-0956-9_5.

Interspecific crossability as a mean to improve Mediterranean sage species with ornamental value



A.N. Martini*, E.G. Stylias, M. Papafotiou, A. Kalantzis and K.F. Bertsouklis

Laboratory of Floriculture and Landscape Architecture, Department of Crop Science, Agricultural University of Athens, Greece

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
ανάπτυξη - εργασία - αλληλεγγύη
Partnership Agreement 2014 - 2020

Co-financed by Greece and the European Union

LUH 2021
VIII International Conference on Landscape and Urban Horticulture

ISHS

OrgHort 2020

INTRODUCTION

Interspecific hybridization is a common and important evolutionary mechanism in plants, which is used by plant breeders in order to create new plant forms or broaden genetic variability.

Aim of the present study, through the proposed crossings, to utilize particular characteristics of five *Salvia* species native to Greece, such as:

- ✓ the bright flower color of *S. pomifera* spp. *pomifera*,
- ✓ the rich early flowering of *S. fruticosa*,
- ✓ the low height of *S. ringens* and *S. tomentosa*,
- ✓ the minimum water requirements of *S. pomifera* and *S. fruticosa*,

in order to obtain hybrids with desirable ornamental and commercial characteristics.

MATERIALS AND METHODS

S. officinalis and *S. fruticosa* were not crossed with each other, because there have already been several hybrids of them in the market.

S. officinalis	*S. ringens*	2019 & 2020
	S. pomifera ssp. pomifera	
	S. tomentosa	
S. fruticosa	*S. ringens*	2020
	S. pomifera ssp. pomifera	
	S. tomentosa	

Each clone was used both as a pollen and seed parent for all crossings.

Pollinations number

- at least 100 flowers for each interspecific crossing
- 50 flowers in self-pollinations

RESULTS AND DISCUSSION

Table 1. Interspecific crosses and self-pollinations of *S. officinalis* (average data of crosses 2019 and 2020).

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

Table 2. Interspecific crosses and self-pollinations of *S. fruticosa* (data of crosses 2020).

Cross	Pollinations number	Successful crosses (%)	Total seed number	Seedling number
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

CONCLUSIONS

- Crossability among the five selected *Salvia* species native to Greece and the eastern Mediterranean was low in most crosses.
- However, a few interspecific hybrids with desirable ornamental characteristics were selected for further experimentation.

S. fruticosa* × *S. ringens

S. officinalis* × *S. ringens

S. officinalis* × *S. pomifera

S. officinalis* × *S. tomentosa

www.salvia-breed-gr.com

Email: martini_agr@yahoo.com