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Genetic Affinities and Essential Oil Composition of *Salvia officinalis* L., *S. fruticosa* Mill., *S. tomentosa* Mill. and their Hybrids

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In order to clarify the relationship between *Salvia officinalis* L., *S. fruticosa* Mill., and *S. tomentosa* Mill., interspecific crosses were made and were followed by a study of chromosome behaviour, pollen fertility, seed set, and essential oil composition of parents and F1 plants. The data presented show that although the three tested *Salvia* species have low crossability they are closely related.

KEY WORDS *Salvia officinalis* L. *S. fruticosa* Mill. *S. tomentosa* Mill. Crossability Fertility Seed set Essential oil Thujones Camphor

INTRODUCTION

The *Salvia officinalis* group (family Labiatae) consists of eight to ten perennial species which are distributed in the Mediterranean Basin and the Near East. Three prominent species in this group are *S. officinalis* L., *S. fruticosa* Mill. (syn *S. triloba*),[1,2] and *S. tomentosa* Mill. *S. officinalis* (sage) is economically the most important species, and is harvested from protected wild populations in its centre of distribution, the Dalmatian area of Yugoslavia. *S. fruticosa* (three-lobed sage) grows in dry, warm Mediterranean garigue formation, from were it is collected, mainly for tea and as a substitute for sage. *S. tomentosa* is found in the cooler northern fringe of the Mediterranean basin, and has local uses.[1-4]

Cytogenetic surveys of the genus *Salvia* are rare and have concentrated mainly on determination of chromosome numbers.[3,5]

In this paper we present the results of the cytogenetic affinities amongst the three species. These include chromosome behaviour, crossability, pollen fertility, seed set, and essential oil composition.

MATERIALS AND METHODS

Parent Plants

Different clones propagated by stem cuttings,[6] were raised from seed collected in natural stands in Israel, Greece and Yugoslavia. These clones were obtained after several years of single plant selection.[7,8] Altogether, six clones, two per each species, including seven plants for every clone, were grown in 10-litre pots (one plant per pot).

Hybridization

Crosses were made between clones from different species by emasculation and manual pollination. The emasculation was done by removal of the anthers, 1-2 days before their dehiscence. All the older flowers (before emasculation) were removed, and one of the calyx pawl in the flower used for hybridization was also removed. The emasculated flowers were pollinated twice, 1 and 2 days after emasculation. The flowers were bagged in 20 ×

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30 cm paper envelopes, from emasculation until seed set formation.

S. tomentosa was used only as a female parent, in crosses with the two other species, because the flowering starts in this species when it is almost finished in the other two.

Crossability was calculated as the percentage of flowers that produce seeds out of the total flowers that were emasculated and pollinated.

Chromosome Counts

Chromosome counts were made during meiosis, by the squashing technique, with aceto-carmine (2%) after fixation in alcohol-acetic acid (3:1) for 24 hours.

Pollen Fertility

Pollen fertility was determined by pressing mature anthers in 4% cotton blue in lactophenol. At least 300 pollen grains per plant were rated and pollen grains were considered normal when they were rounded and well stained.

Essential Oil

The essential oil was hydrodistilled from fresh plants in a modified Clevenger apparatus for 2 h at 100°C. From the cooled essential oil, a neat sample of 0.1 μl was analysed on a Varian 3700 gas–liquid chromatograph, with a flame ionization detector a Hewlett-Packard 3390 integrator. The column was 5% Carbowax 20M on Chromosorb W 80/100 mesh, 3 m × 4 mm i.d., with a gas flow of 30 ml N_2/min held at 70°C for 2 min, and then programmed to 200°C at 6°C/min and held at 200°C for 4 min.

RESULTS AND DISCUSSION

The parents were found to be cross-compatible, and gave a sizable yield of hybrid seeds (Table 1). This was obtained in crosses between *S. officinalis* and *S. fruticosa*, but when *S. tomentosa* was crossed with these two species the crossability was low; zero to 2% in the cross with *S. officinalis*, and zero in the cross with *S. fruticosa* where *S. tomentosa* was used as the male parent. A large number of these hybrid seeds did not germinate, or when they developed normally, some died at the seedling stage, while others did not reach flowering.

Meiosis in both parents and the surviving hybrids was normal, showing regular formation of seven bivalents. In the hybrids, high rates of abortion (50%–70%) were found in the tetrad cells at the end of meiosis, while the parents showed only a 10% to 15% cell loss.

A significant level of pollen sterility and seed set loss was found in all hybrid combinations. While parent pollen fertility ranged between 80% and 90%, in hybrids it amounted to no more than 16% (Table 1). A similar situation was encountered in seed set, where 30% to 70% of the parent ovules developed into seeds while only 5% to 15% of the F1 hybrids ovules did so. From these results it was concluded that hybrid inviability and sterility reproductive barriers exist among the three tested *Salvia* species and that this partly bars gene-exchange between them.

The essential oil of *S. fruticosa* is characterized by 1,8-cineole (48%), but contains also, camphor, β-caryophyllene, α-pinene and β-pinene. α- and β-thujone were not detectable (Table 2).[9]

The main component in the oil of *S. officinalis* is α-thujone (55%), the others are β-thujone and 1,8-cineole, while α- and β-pinene, β-caryophyllene and camphor are present in low levels.[8]

Table 1. Crossability, viability, pollen fertility and seed set of three *Salvia* species and their hybrids

Parent	Crossability (%)	Viability of F1 (%)	Pollen fertility (%)	Seed set (% in open pollination)
S. officinalis	85	93	90	65
S. fruticosa	92	90	88	37
S. tomentosa	96	94	87	40
S. officinialis × *S. fruticosa*	36	38	16	9
S. fruticosa × *S. officinalis*	34	35	10	9
S. tomentosa × *S. officinalis*	2	47	15	4
S. tomentosa × *S. fruticosa*	21	0[a]	0	0

[a]Germination 100%.

Table 2. Percentage of main components in the essential oil of three *Salvia* species and their hybrids.

Parents and hybrids	Main components (%) in the essential oil							
	Pinene		Myrcene	1,8-Cineole	Thujone		Camphor	Caryophyllene
	α	β			α	β		
S. officinalis	1	1	2	13	55	10	2	0
S. fruticosa	6	11	4	48	0	0	8	8
S. tomentosa	2	2	5	19	0	59	1	0
S. officinalis × *S. fruticosa*	3	7	3	30	27	7	4	4
S. fruticosa × *S. officinalis*	3	7	3	24	29	7	4	2
S. tomentosa × *S. officinalis*	2	3	6	12	49	6	4	2

β-Thujone is the main component in the oil of *S. tomentosa* while α-thujone was not detected.[4]

In the essential oils of the hybrids between *S. fruticosa* and *S. officinalis* (regardless of which is the maternal or paternal parent), there is a medium level of 1,8-cineole, α- and β-thujone, camphor, β-caryophyllene and α- and β-pinene. The GC chromatogram of the oil of the hybrid between *S. tomentosa* and *S. officinalis* is very similar to that of *S. officinalis* and the concentration of β-thujone is not increased because of the influence of *S. tomentosa* (Table 2). The hybrids resembled *S. officinalis* in the level of thujones, but 1,8-cineole and camphor levels were intermediate between the parents.

The data presented show that the three tested *Salvia* species, although having low crossability, are still closely related. Moreover, they are reproductively isolated primarily not by genetic, but by geographic and ecological barriers, and by different flowering periods.

Because the interspecific hybrids between the examined salvias are partly fertile, it is already possible to recommend the use of *S. fruticosa* and *S. tomentosa* as genetic sources for sage breeding. It seems highly possible that other wild species in the *S. officinalis* group are also not isolated from the cultivated spice by complete inviability or sterility barriers. In the future it will probably be possible to utilize also the gene pools of these wild species.

In conclusion, there exists a large yet unexploited gene pool in the *S. officinalis* group which could be used to develop new breeds of sage suited to specific agronomic conditions, and containing desired combinations of essential oils.

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