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Genetic investigation of natural hybridization between *Salvia officinalis* L. and *Salvia fruticosa* Mill. on the island of Vis

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Abstract

Dalmatian sage (*Salvia officinalis* L.) and Greek sage (*Salvia fruticosa* Mill.) grow sympatrically on the island of Vis. In Croatia, *S. fruticosa* is found only on the island of Vis where it forms small isolated populations in the vicinity of Komiža, whereas *S. officinalis* is distributed throughout eastern Adriatic coastal region. Detailed morphological analysis revealed the existence of morphologically intermediate individuals that indicated natural hybridization between the two species. Until now, spontaneous hybrids of the species were not observed, although their hybrid derived from artificial crossing has been described as *Salvia* x *auriculata* Mill. In order to confirm natural hybridization between *S. officinalis* and *S. fruticosa* on genetic level, SSR and AFLP markers have been utilized. The obtained results strongly supported the ongoing hybridization events. Our findings will not only contribute to conservation efforts but will also provide a solid foundation for future plant breeding programs and agricultural exploitation.

Keywords

Amplified Fragment Lenght Polymorphism (AFLP),
Hybridization,
Salvia officinalis L.,
Salvia fruticosa Mill.,
Simple Sequence Repeats (SSR)


Fig. 1. *S. officinalis* L.


Fig. 2. *S. fruticosa* Mill.


Fig. 3. *S.* x *auriculata*

Introduction

Salvia officinalis L. and *Salvia fruticosa* Mill. are the species of Lamiaceae family used from ancient times in traditional medicine. *Salvia officinalis* L. contains numerous biologically active compounds with gastro-protective, anti-diabetic, anti-inflammatory and bactericidal effects (Hamidpour et al., 2014 and references herein). The essential oil of *S. fruticosa* exhibits antibacterial, cytotoxic and antiviral activities (Sivropoulou et al., 1997) and it is a folk remedy for toothaches and intestinal disorders (Rivera et al., 1994).

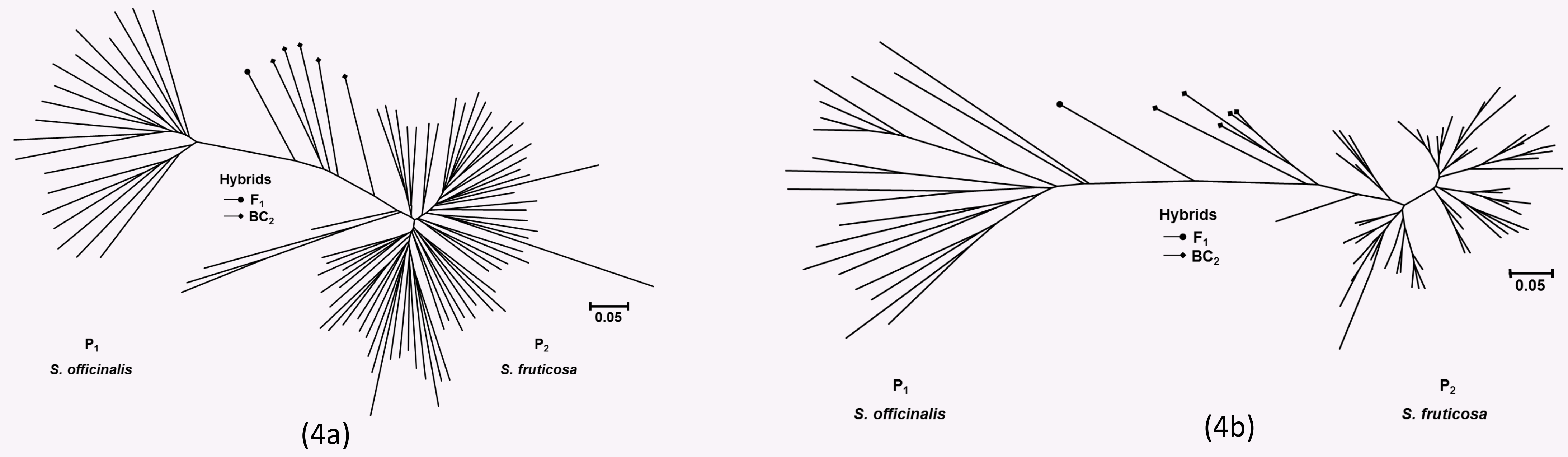
On the Island of Vis *S. officinalis* and *S. fruticosa* grow in the same habitat with overlapping flowering periods in late April and early May. The species differ in morphological characteristics, however, they have the same number of chromosomes (2n=14) (Karousou et al., 2000) and pollinator assemblage (Corsi and Bottega, 1999).

The aim of our research was to confirm the hypothesis of the existence of natural hybridization on the island of Vis using two complementary molecular methods SSRs and AFLPs.

Materials and methods

Genomic DNA was isolated from 95 individuals with DNA-GenElute Plant Genomic DNA Miniprep Kit (Sigma Aldrich, Steinheim, Germany), according to manufacturer's instructions. The individuals were sampled and classified as *S. officinalis*, *S. fruticosa* or their hybrids on the basis of their morphological characteristics. Seven microsatellite loci previously developed for *S. officinalis* (Radosavljević et al., 2011) and four AFLP combinations were used for genotyping.

On the basis of AFLP marker data Dice's distance coefficients between individual plants were obtained as 1-Dice's similarity index (Dice, 1945). A Neighbour-Joining tree based on Dice's distance matrix was constructed. The calculations were made using PAST version 2.01 (Hammer et al., 2001). Genetic distances between pairs of samples were calculated based on microsatellite marker data using the proportion-of-shared-alleles distances (D_{psa}; Bowcock et al., 1994) as implemented in MICROSAT (Minch et al., 1997). Cluster analysis was performed using the Neighbour-Joining method as implemented in NEIGHBOR programme of the PHYLIP ver. 3.6b software package (Felsenstein, 2004). A model-based clustering method was applied to infer genetic structure and define the number of clusters using the software STRUCTURE ver. 2.3.3 (Pritchard et al., 2000). The Bayesian method implemented by NewHybrids 1.1. (Anderson and Thompson, 2002) was used to assign individuals into six classes: two parental (P_1 *S. officinalis* and P_2 *S. fruticosa*) and four hybrid (F_1, F_2, and backcrosses with the parental populations) .



(4a) (4b)

Fig. 4. Neighbour-Joining tree of *Salvia officinalis*, *S. fruticosa* and hybrids based on (a) AFLP and (b) SSR marker data. Hybrids are designated as F_1 or BC_2 as revealed using NewHybrids.

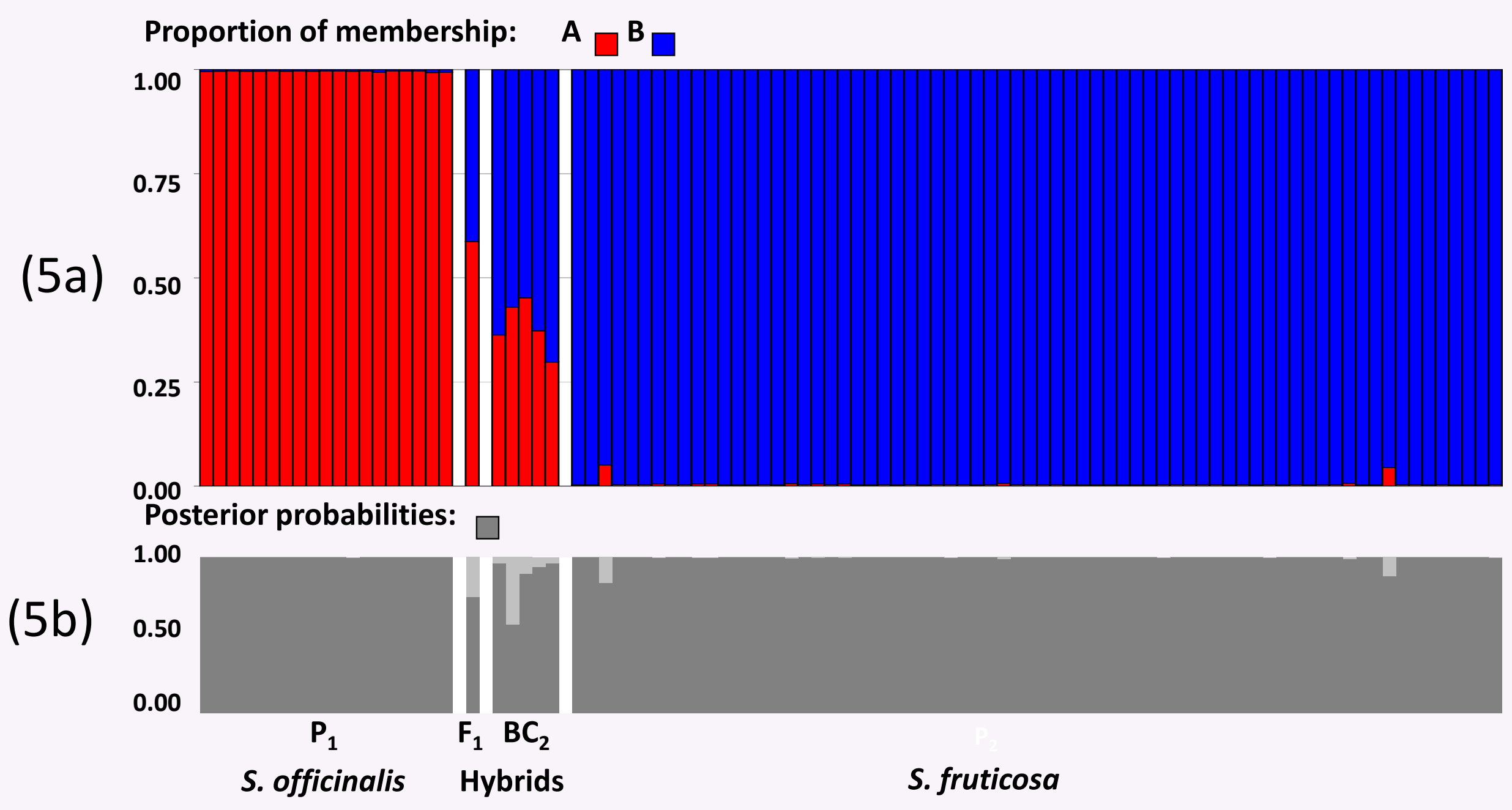


Fig. 5. Assignment of *Salvia officinalis*, *S. fruticosa* and hybrid individuals into (a) genetic clusters as estimated by the program STRUCTURE and (b) classes (parental individuals, F_1 and BC_2) as estimated by the program NewHybrids

Results and discussion

The NJ trees on the basis of both AFLP and SSR marker data revealed the existence of three groups of individuals (Fig. 4a and 4b). The STRUCTURE analysis indicated K=2 as the most likely number of clusters. The two genetic clusters corresponded to the two *Salvia* species, while six individuals of admixed origin were detected (Fig. 5a). The hybrid assignment using NewHybrids was congruent with STRUCTURE as all the individuals characterized by admixed proportions of membership were confirmed to be of hybrid origin. A single individual was classified as F_1 hybrid and five individuals were classified as back-crosses of *S. fruticosa* (BC_2) (Fig. 5b).

Conclusion

Molecular analysis confirmed natural hybridization between *S. officinalis* and *S. fruticosa* on the island of Vis. Natural hybrids represent a potential value for agronomy, pharmaceutical, food and cosmetic industry, therefore, future research should be aimed at chemical analysis of the essential oil and the evaluation of their potential for future breeding programs.

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