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Short Communication

Assessment of genetic diversity within and among sage (*Salvia*) species using SRAP markers

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Abstract

Salvia (sage) is the most important and largest genus of the *Lamiaceae* family. High similarities among species in this genus lead to difficulty in its systematic identification. Despite its economic importance, limited molecular studies have been conducted to evaluate the genetic diversity among and within *Salvia* species. In this study, SRAP (sequence-related amplified polymorphism) markers, which targeted ORFs (open reading frames) as functional regions in the genome, were used to detect the genetic diversity of five *Salvia* species (*S. virgata* Jacq., *S. nemorosa* L., *S. officinalis* L., *S. cereal* L. and *S. sclarea* L.). Fourteen primer combinations (PCs) were amplified by 265 fragments on 54 genotypes, in which 255 (96%) were polymorphic. The average polymorphism information content (PIC) value was 0.308 over all PCs. The genetic distance among species ranged from 0.126 (between *S. virgata* Jacq. and *S. nemorosa* L.) to 0.568 (between *S. nemorosa* L. and *S. sclarea* L.). Based on Jaccard's similarity coefficient and UPGMA algorithm, cluster analysis separated different species ($r = 0.920$). The results showed high genetic differentiation ($F_{st} = 0.337$) and negligible gene flow ($N_m = 0.750$) among species. Owing to the high genetic variation among and within *Salvia* species, it serves as a rich source of germplasm with potential for use in breeding programmes.

Keywords: AMOVA; genetic variation; *Salvia*; SRAP

Introduction

Salvia is an important and also the largest genus of the *Lamiaceae* family, which includes nearly 1000 species (Walker and Sytsma, 2007). Fifty-eight annual or perennial species of the genus have been found in Iran, 17 of which are endemic (Walker and Sytsma, 2007). Some of the *Salvia* species are considered as a valuable spice in food industries (Gali-Muhtasib *et al*., 2000), and grown in parks and gardens as ornamental plants.

Identification of *Salvia* species is complicated due to the morphological similarity and common occurrence of natural hybridization within species (Reales *et al*., 2004; Walker *et al*., 2004). Molecular markers have been widely used in the identification of species and genotypes (Skoula *et al*., 1999; Karaca *et al*., 2004, 2008; Bertea *et al*., 2005). Numerous SSR markers have also been developed for the most important *Salvia* species, *S. officinalis* L. (Mader *et al*., 2010;

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Radosavljević *et al.*, 2011, 2012; Wang *et al.*, 2011). Among the different molecular marker systems, sequence-related amplified polymorphism (SRAP) is a relatively simple and highly reproducible DNA marker (Li and Quiros, 2001).

Within the genus *Salvia*, there are few species of significant economic importance, and numerous studies have focused on cultivation, effective ingredients and pharmacological properties of these species (Gali-Muhtasib *et al.*, 2000; Delamare *et al.*, 2007; Kelen and Tepe, 2008). According to the literature, no report has been recorded regarding genetic diversity at the inter-specific level among *Salvia* species using SRAP markers. Therefore, this study aimed at the utilization of SRAP markers in assessing the genetic diversity of *Salvia* species including *S. virgata* Jacq., *S. nemorosa* L., *S. officinalis* L., *S. sclarea* L. and *S. cereal* L.

Experimental procedure

Aerial parts of 54 sage samples belonged to *S. virgata* (12), *S. nemorosa* L. (14), *S. officinalis* L. (15), *S. sclarea* L. (3) and *S. cereal* L. (10) species, which were collected from different regions in Iran (Supplementary Table S1 and Fig. S1, available online). Genomic DNA was extracted from the ground powder using a HiYield genomic DNA mini kit (HiYield™ Genomic DNA Mini Kit, Real Biotech Corporation, Banqiao City, Taiwan) following the manufacturer's instructions.

PCRs and amplifications were performed according to Li and Quiros (2001). The amplified products were separated on 8% non-denatured polyacrylamide gel electrophoresis and visualized by silver staining (Bassam *et al.*, 1991).

Based on the presence of reproducible polymorphic bands on the gel, DNA fragments were scored in all the 54 *Salvia* species samples. A dendrogram was constructed based on Jaccard's similarity coefficient using the UPGMA (unweighted pair group method with arithmetic mean) algorithm, and genetic relationships among genotypes were further analysed by the principal coordinate analysis (PCoA) of a similarity matrix using NTSYS-pc version 2.02 (Rohlf, 1998).

The PIC was calculated for all selected markers according to Smith *et al.* (1997).

Genetic diversity within and among species was measured by the percentage of polymorphic bands, the effective number of alleles, the observed number of alleles, Nei's gene diversity, Shannon's information index and gene flow. The UPGMA dendrogram of species was constructed using NTSYS-pc version 2.02,

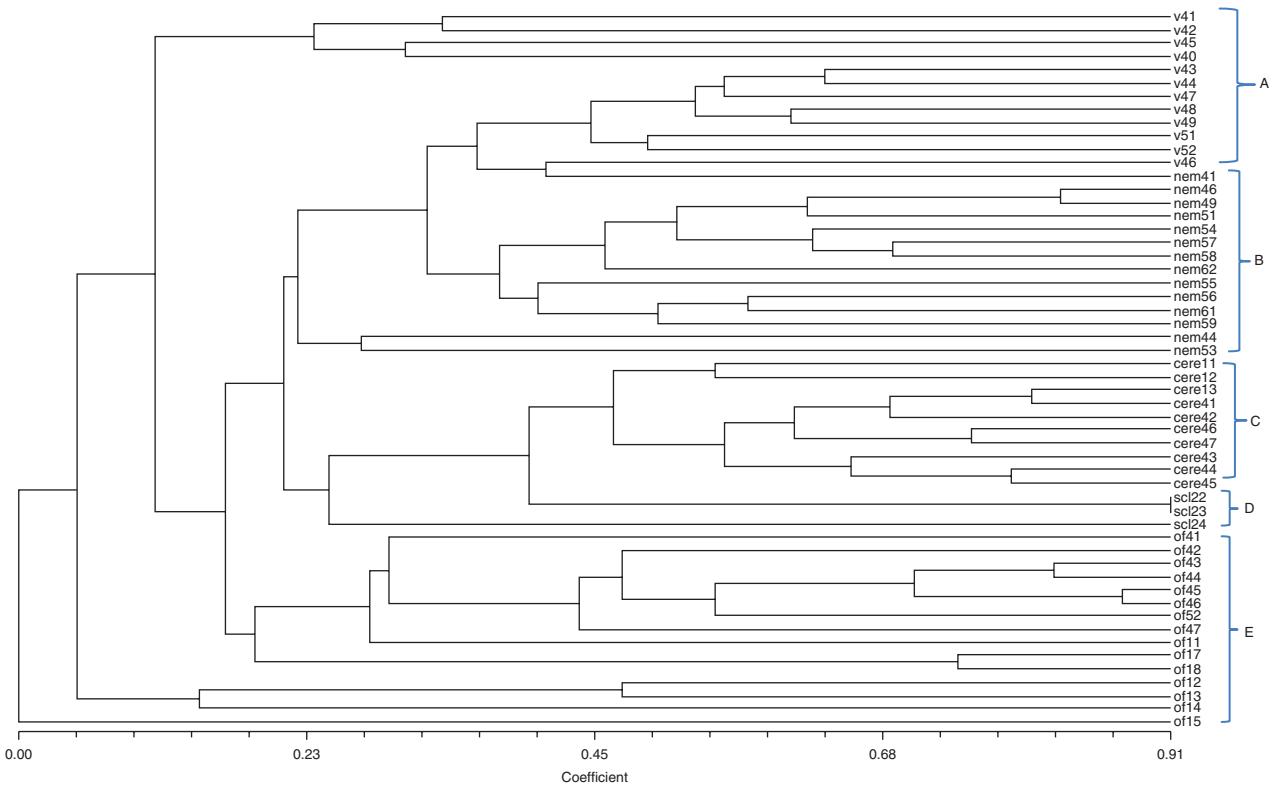

Fig. 1. UPGMA dendrogram of 54 sage genotypes using SRAP markers based on Jaccard's coefficient. A, B, C, D and E groups consist of *S. virgata, S. nemorosa* L., *S. cereal* L., *S. sclarea* L. and *S. officinalis* L., respectively.

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Table 1. Summary of genetic variation statistics for all loci in the five *Salvia* species

Species name	Sample size	NPB	PPB	H_e	I	N_a	N_e
S. virgata	12	162	63.53	0.221	0.332	1.635	1.381
S. nemorosa	14	177	69.41	0.251	0.375	1.694	1.429
S. cereal	10	128	50.20	0.181	0.270	1.502	1.307
S. sclarea	3	27	10.59	0.090	0.129	1.203	1.162
S. officinalis	15	137	53.73	0.210	0.307	1.537	1.376

NPB, number of polymorphic bands; PPB, percentage of polymorphic bands; H_e, Nei's gene diversity; I, Shannon's information index; N_a, observed number of alleles; N_e, effective number of alleles.

and based on co-ancestry coefficients obtained from the pairwise F_{st} distance matrices using POPGENE 1.32 software (Yeh and Yang, 1999).

Results and Discussion

A total of 32 different SRAP primer combinations (PCs) were evaluated for their ability to prime the PCR amplification of five randomly selected sage samples from different species. Fourteen selected PCs were amplified by 265 bands among the 54 *Salvia* species samples, of which 255 bands were polymorphic (96%). The high polymorphism rate found in this study is in agreement with the previous observations of genetic diversity among *Salvia* species (Song *et al*., 2010; Sepehry Javan *et al*., 2012; Zhang *et al*., 2013; Peng *et al*., 2014). The mean PIC value for the PCs was 0.308, which ranged from 0.201 to 0.394 (Supplementary Table S2, available online), indicating that SRAP markers showed medium polymorphism and could contribute to the genetic variation of *Salvia* species.

The UPGMA dendrogram with a high cophenetic correlation coefficient ($r = 0.920$) based on SRAP markers grouped the five species into five distinct clusters (Fig. 1), which is in agreement with the previous observations of Sepehry Javan *et al*. (2012). The A, B, C, D and E groups consisted of *S. virgata*, *S. nemorosa* L., *S. cereal* L., *S. sclarea* L. and *S. officinalis* L., respectively. The SRAP analyses showed *S. virgata* Jacq. and *S. officinalis* L. as the most divergent ones.

Genetic relationships among the sage samples were also analysed by the PCoA. The first three principal coordinates explained 26.2% of the total variation, showing that the original data were not highly correlated in the PCoA.

The 54 sage samples were grouped into five groups by their species. On the whole, among the five species, the highest Shannon's information index, Nei's gene diversity index, the observed and effective number of alleles, and the percentage of polymorphic loci were found in *S. nemorosa* L. (Table 1). The largest genetic distance (0.565) was observed between *S. nemorosa* L. and *S. sclarea* L. and the smallest one (0.126) occurred between *S. nemorosa* L. and *S. virgata* Jacq. (Supplementary Table S3, available online).

In conclusion, SRAP markers, which targeted ORFs (open reading frames) as functional regions of the sage genome and reached the resulting sufficient polymorphism, can be successfully used for determining the genetic diversity and population structure of *Salvia* species.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1479262115000593

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