RAPID BASED GENETIC DIVERSITY AMONG SALVIA OFFICINALIS L. POPULATIONS

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Abstract

Four Salvia officinalis L. populations were examined for the extent of genetic variability and compared with S. judaica Boiss., by extracting genomic DNA and generating a random amplified polymorphic DNA (RAPD) marker profile. Hierarchical cluster analysis separated the common sage populations into two groups. The two Hungarian S. officinalis L. samples were tightly clustered (61% dissimilarity). The Greek and Romanian populations also clustered together, and were genetically more distinct from the Hungarian samples (77% dissimilarity). The S. officinalis L. group, including all populations, was separated from S. judaica Boiss. with the greatest genetic distance (83% dissimilarity).

INTRODUCTION

Salvia officinalis L., one of the most important representatives of Lamiaceae family, has been known for its medicinal and culinary uses since ancient times. As a medicinal herb, it is valued because of its antiseptic, anti-inflammatory, antioxidant, carminative, cholagogue and diaphoretic properties. Common sage is widespread in the Mediterranean, South-East Africa, as well as in Central and South America, where it is largely
cultivated both for culinary and medicinal purposes. A broad range of applications is known in aromatherapy and food industry.

Both the quantity and composition of the essential oil may differ among various sages or even within species, which may reflect either environmental or genetic differences or both. The investigations on *S. fruticosa* Mill. populations in Crete suggested that the basis of variation in the essential oil composition depends more on the genetic background and less on climatic variations found slightly greater genetic diversity among wild varieties of *S. hispanica* L. in comparison to domesticated varieties [1, 4].

The present study aimed at determining the genetic diversity among four European populations of common sage, analyzing their genetic profiles using random amplified polymorphic DNA (RAPD) markers. Random amplified polymorphic DNA (RAPD) markers have been used in a wide range of plant species for cultivar identification and assessment of genetic relationships, especially at lower intraspecific taxonomic levels [2, 3, 6].

**MATERIALS AND METHODS**

**Plant material**

Leaf samples of *S. officinalis* L. populations originated from Greece (Salonik), Romania (Bihor) and Hungary. The first Hungarian sample was a commercial sage leaf tea, whereas the other Hungarian sage sample, together with leaves of *S. judaica* Boiss. (used as outgroup) were collected from the botanic garden of the University of Pecs, Hungary, in 2006 and 2007.

**Isolation of genomic DNA**

100 mg of leaf samples was ground in liquid nitrogen and extraction proceeded by using a QIAGEN DNeasy plant mini kit.

**Polymerase chain reaction (PCR) amplification**

PCR reactions were performed in a total reaction volume of 12 µl. The PCR mix contained 25mM MgCl₂, 10 x buffers, 100 mM each dATP, dCTP, dGTP and dTTP and distilled water. Altogether 59 random decamer oligonucleotide primers (Operon Technologies) were used for amplifications. Reaction mixtures were amplified in a PTC-200 thermal cycler (Perkin-Elmer, USA). The thermal cycle used was 2 min at 94°C, followed by 36 cycles of 10 sec at 94°C, 30 sec at 36°C, 1 min at 72°C. A final cycle of 2 min at 72°C completed extension of remaining products prior to holding the samples at 4°C for analysis. Amplified fragments, along with a 100 bp DNA ladder (Fermentas) were separated by electrophoresis on horizontal 1.5% agarose gels in 0.5x Tris-Borate-EDTA (TBE) buffer. Gels were stained with ethidium bromide, visualized under UV light and photographed using a BioDoc-It™ System UV Transilluminator (UVP Inc., California).
Data analysis

Bands were scored as a binary variable, 1 for presence and 0 for absence of a band. Only distinct, well-resolved, stable bands were considered. Band-sharing analysis was conducted, using the Jaccard’s coefficient. A dendrogram was constructed using the unweighed pair group method with arithmetical average (UPGMA) to estimate relationships among sage taxa. The software SYN-TAX 2000 was used to perform the cluster analysis.

RESULTS AND DISCUSSION

Four *Salvia officinalis* L. populations were examined for the extent of genetic variability and compared with *S. judaica* Boiss., by extracting genomic DNA and generating an RAPD marker profile. From 59 primers of kits A, B, N and O of Operon Techn., 9 oligonucleotide primers were selected for the evaluation of relationships. Altogether 259 RAPD bands were scored, ranging in size from 150 to 1500 bp. The number of amplification products per primer varied from 1 to 8. Including *S. judaica* Boiss. in the analysis, 27 (10%) of the bands were polymorphic. Hierarchical cluster analysis of the taxa, using RAPD markers, produced a dendrogram of genetic relatedness as shown in fig.1. The level of polymorphism observed within *S. officinalis* L. demonstrates genetic variation between populations. Cluster analysis separated the populations into two groups.

The two Hungarians *S. officinalis* L. samples are tightly clustered, with the lowest (61%) dissimilarity value, indicating that they are genetically more related. This is not unexpected, since these populations were also the closest geographically. The Greek and Romanian populations also cluster together, and they are genetically more distinct from the Hungarian samples (77% dissimilarity of the two clusters). The *S. officinalis* L. group, including all populations, was separated from *S. judaica* Boiss., as expected, and the genetic distance was the greatest between the *S. officinalis* L. cluster and *S. judaica* Boiss. (83% dissimilarity).

Skoula et al [4] observed in *S. fruticosa* Mill. clones that the genetic profiles generated by RAPDs corresponded to the patterns of relatedness in chemical profiles, suggesting that there may be a genetic basis for the chemical profiles observed. Vieira et al. [5] found that although RAPDs are markers obtained at random through the whole plant genome, and do not reflect necessarily any specific morphological or chemical trait, RAPD markers were strongly correlated to volatile oils and flavonoids of *Ocimum gratissimum* L., and were able to distinguish the three chemotypes in this species. The correlation between the molecular markers and volatile oils indicates that molecular markers can be found linked to their volatile oil
constituents. Our preliminary studies on the genetic diversity of common sage may also provide a base for future basic and applied research related to \textit{S. officinalis} L. In further studies, we plan to compare the essential oil content and composition of various \textit{S. officinalis} L. populations and reveal if these findings can be correlated with genetic diversity based on RAPD markers.

\textbf{Figure 1}
Hierarchical cluster analysis of the taxa using RAPD markers
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REFERENCES