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Evaluation of genetic diversity in *Salvia* based on RAPD marker

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Abstract: *Genus of Salvia is one of the widely distributed plants in the world and comprises 58 species in Iran. Using RAPD molecular marker, genetic variation between eight species of Salvia was investigated. The samples were collected from Iran various locales. 20 RAPD primers produced 217 scorable bands which 213 bands were polymorphic (98/2%). The pair-wise genetic distance varied from 0.056 to 0.588. The dendrogram was constructed using UPGMA method with the help of NTSYSpc 2.02i software, which distinguished seven main groups among eight species of Salvia. In the present study, detected polymorphism level represents high genetic variation at interspecies level in Salvia genus.*

Keywords: *Primer, dendrogram, RAPD, Salvia, UPGMA.*

INTRODUCTION

The genus *Salvia*, as the largest genus in the family of Lamiaceae, consists of various annual and perennial herbaceous plants with more than 900 species (Saeidnia et al., 2009). This genus has a high environmental compatibility (Song et al., 2010) and has spread throughout the world, especially in tropical and temperate regions (Delamare et al., 2007). It includes 58 species in Iran (Saeidnia et al., 2009), from which 17 species are endemic for Iran (Mozaffarian, 1996). This genus is distinguished from other Lamiaceae's species by its unusual structure of stamen (Walker et al., 2004). *Salvia* seeds have nutritioval, medicinal and oil value (Delamare et al., 2007). The term *Salvia* means medicine in Latin, and this genus has a historical reputation for health and cure of diseases (Keller et al., 2005). The *Salvia* extract has antibiotic, antibacterial, antitumor, antioxidant and anti-diabetic activities that treat various types of diseases (Yan and Wang, 2007). The effect of environmental and genetic factors has been shown on *Salvia* essential oils (Delamare et al., 2007). The three dominant factors affecting the genetic diversity of *Salvia* include the species, geographical range, and human selection (Cahill, 2004). Molecular markers provide a powerful tool for description and management of germplasm. DNA fingerprinting using RAPD is widely used to find the polymorphism of medicinal plants (Ramesh et al., 2011). The RAPD technique has vastly expanded due to advantages such as the unlimited number of strips, the lack of use of radioactive material, its simplicity and the speed of its function for assessing genetic diversity and phylogenetic relationships (Powell et al., 1996). Following the first research on the study of genetic relationships among eight *Salvia* species with molecular markers RAPD and ISSR

(Sepehry et al., 2011), this is the second study in Iran among eight other *Salvia* species using the RAPD molecular marker.

Materials and Methods

In this study, the leaves of eight species of *Salvia* were collected from different areas of Iran and transferred to freezer with -80 ºC in liquid nitrogen. Extraction of genomic DNA from the leaves of plants was performed using CTAB method (Doyle and Doyle, 1987). The concentration of DNA was determined by spectrophotometry and genomic DNA was transferred to a freezer with temperature of -20 °C. Polymerase chain reaction (PCR) was performed on Applied Biosystem. PCR products were isolated on 2% agarose gel to observe the banding pattern. The scoring of bands was performed as 1 and 0 for presence and absence of the band, respectively, in order to analyze data. Data analysis was performed with NTSYSpc 2.02 software.

Results and Discussion

The RAPD technique has already been used to determine the genetic variation of many plants such as wheat (Suvarna, 2001), barley (Iidiko Karsai et al., 2006), and pomegranate (Sarkhosh et al., 2006). The results show that this marker is very useful for evaluating interspecies genetic variation. This research was conducted in following the first research to investigate genetic relationships among eight *Salvia* species in Iran with molecular markers RAPD and ISSR (Sepehry et al., 2011). Accordingly, genetic relationships between eight other *Salvia* species were determined using the RAPD molecular marker. Twenty randomized RAPD primers were used, which all primers produced clear and polymorphic bands (Table 1).

Table 1: The titles and sequence of the primers used in PCR

Primer	Number of generated bands	Number of polymorphic bands	Polymorphism percent	Sequence
OPA-01	11	11	100	5'-CAGGCCCTTC-3'
OPA-02	7	7	100	5'-TGCCGAGCTG-3'
OPA-03	9	9	100	5′-AGTCAGCCAC-3′
OPA-04	10	10	100	5′-AATCGGGCTG-3′
OPA-05	11	11	100	5′-AGGGGTCTTG-3′
OPA-06	12	12	100	5′-GGTCCCTGAC-3′
OPA-07	10	10	100	5′-GAAACGGGTG-3′
OPB-01	11	11	100	5′-GTTTCGCTCC-3′
OPB-02	10	10	100	5′-TGATCCCTGG-3′
OPB-03	12	12	100	5′-CATCCCCCAG-3′
OPB-05	13	13	100	5′-TGCGCCCTTC-3′
OPC-04	10	10	100	5'-CCGCATCTAC-3'
OPD-02	11	11	100	5′-GGACCCAACC-3′
OPD-03	8	8	100	5′-GTCGCCGTCA-3′
OPD-05	9	9	100	5′ -TGAGCGGACA-3′
OPD-08	11	11	100	5′-GTGTGCCCCA-3′
OPD-11	14	14	100	5′-AGCGCCATTG-3′
OPE-08	10	9	90	5'-TCACCACGGT-3'
OPM-17	12	9	75	5'-TCAGTCCGGG-3'
OP0-15	16	16	100	5'-TGGCGTCCTT-3'
Total	217	213	98.2	

The results obtained with the OPM-17 primer are shown in Figure 1. The 2017 accountable bands were created by RAPD primers in PCR, of which 213 bands were polymorphic bands (98.2%). The results showed that there is a high level of genetic variation among *Salvia* species. The genetic similarity between *Salvia* species ranged from 0.411 (between *S. macrosiphon* and *S. urmiensis*) to 0.944% (between *S. verbenaca* and *S. oligophylla*) (Table 2).

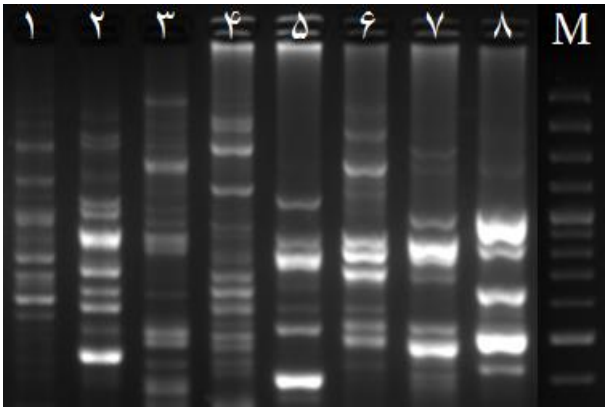

Fig. 1: RAPD profile based on OPM-17 primer. M: DNA ladder in the size of 100-3000, 1 = *S. verbenaca*, 2 = *S. macrosiphon*, 3 = *S. brachyantha*, 4 = *S. aristata*, 5 = *S. urmiensis*, 6 = *S. sahendica*, 7 = *S. bazmanica*, 8 = *S. oligophylla*.

Table 2: Similarity matrix of species based on Jaccard's similarity coefficients

	1	2	3	4	5	6	7	8
S. verbenaca	1.0000							
S. macrosiphon	0.5887	1.0000						
S. brachyantha	0.6277	0.5368	1.0000					
S. aristata	0.6147	0.5152	0.6753	1.0000				
S. urmiensis	0.5541	0.4113	0.6061	0.5498	1.0000			
S. sahendica	0.4719	0.5281	0.4372	0.4675	0.4502	1.0000		
S. bazmanica	0.6061	0.5498	0.5455	0.5671	0.4719	0.4675	1.0000	
S. oligophylla	0.9437	0.5844	0.6320	0.6190	0.5498	0.4675	0.6017	1.0000

1 = S. verbenaca, 2 = S. macrosiphon, 3 = S. brachyantha, 4 = S. aristata, 5 = S. urmiensis, 6 = S. sahendica, 7 = S. bazmanica, 8 = S. oligophylla.

The genetic distance of the species varied from 0.056 to 0.588. The dendrogram was constructed using the UPGMA method, as shown in Figure 2. The dendrogram depicted seven main groups among eight *Salvia* species as follows: (1) *S. verbenaca* and *S. oligophylla*, (2) *S. brachyantha*, (3) *S. aristata*, (4) *S. bazmanica*, (5) *S. macrosiphon*, (6) *S. urmiensi*, and (7) *S. sahendica* (Fig. 2).

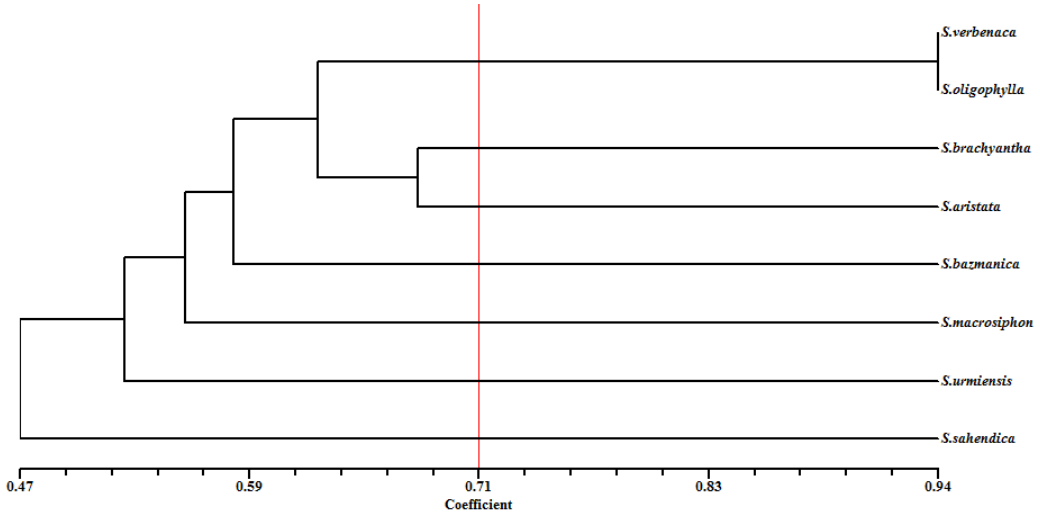

Fig. 2: Dendrogram of the genetic distance of 8 *Salvia* species

The results of this study showed that RAPD molecular markers for analyzing genetic variation and phylogenetic relationships between *Salvia* species were appropriate markers and showed a wide range of variation.

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