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Genetic Diversity Assessment of some *Salvia* sp. Ecotypes Based on ISSR Markers

Masoumeh Yousefiazarkhanian*, Ali Asghari**, Jafar Ahmadi, Behvar Asghari**** and Ali Ashraf Jafari*****

**Ph.D. Student. University of Mohaghegh Ardabili, Ardabil, Iran.*
***Associate Prof. University of Mohaghegh Ardabili, Ardabil, Iran*
****Associate Prof., Imam Khomeini International University, Qazvin, Iran*
*****Assistant Prof., Imam Khomeini International University. Qazvin, Iran*
******Research Prof., Research Institute of Forest and Rangelands, Tehran, Iran.*

(Corresponding author: Masoumeh Yousefiazarkhanian)
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ABSTRACT: The genus *Salvia* L. belongs to the Lamiaceae family with about 1000 species worldwide including 55 species in Iran. This research was conducted to study of genetic variation between and within *Salvia aethiopsis, S. macrosiphon* and *S. virgata* ecotypes by ISSR molecular technique. Five investigated primers could totally produce 65 bands of amplified DNA fragments in which 50 bands (76.9%) revealed polymorphism. The average values of PIC and MI were 0.46 and 3.61, respectively. Cluster analysis by Jaccard similarity coefficient and UPGMA algorithm showed that the ecotypes of each species could group together. On the other hand, cophenetic correlation coefficient (0.99) justified the conversion of Jaccard similarity matrix to dendrogram of cluster analysis. Analysis of molecular variance (AMOVA) indicated that the diversity within the species (18%) was lower than the variation between species (82%). Totally the results stated the ability of species differentiation by ISSR technique.

Key words: AMOVA, Genetic diversity, ISSR, *Salvia*.

INTRODUCTION

The genus *Salvia* (tribe Mentheae, Lamiaceae) represents a cosmopolitan assemblage of nearly 1000 species displaying a remarkable range of variation in three regions of the world: Central and South America (500 spp.), central Asia/Mediterranean (250 spp.), and eastern Asia (90 spp.). This genus includes 58 species in Iran of which 17 are endemic (Sepehry Javan *et al*., 2012 and Walker *et al*., 2004). Molecular markers that show polymorphism at the DNA level have been mentioned as a powerful tool for the estimation of plant genetic diversity (Peng *et al*., 2014) and using these markers especially with emphasizing on proliferation of open reading frames can be effective on genetic diversity and comparative genomic studies (Sepehry Javan *et al*., 2012). ISSR is a simple and efficient marker system for identification of genetic diversity for plant germplasm collection (Peng *et al*., 2014). The efficiency of molecular marker techniques like RAPD and ISSR in *Salvia* species was investigated in terms of genetic diversity and comparative genomics by some authors (Sepehry Javan *et al*., 2012., Song *et al*., 2010 and Wang *et al*., 2011). They reported high polymorphism about these markers in some *Salvia* species and also stated that mentioned techniques can be useful for studying of genetic diversity in *Salvia* (Sepehry Javan *et al*., 2012 and Song *et al*., 2010). ISSR molecular markers have employed to show polymorphisms and distinguish *Salvia miltiorrhiza* germplasms by integrating with phenotypic characteristics (Zhang *et al*., 2013). The aim of present study was to assess the genetic relationship among three species of *Salvia* including nine ecotypes from different regions of Iran using ISSR genetic marker.

MATERIAL AND METHODS

Genomic DNA was extracted from nine ecotypes of three species named *Salvia. aethiopsis, S. macrosiphon* and *S. virgata* as described by Piccolo *et al*., 2012. The quality and quantity of DNAs were evaluated by 0.8% agarose gel electrophoresis and nanodrop spectrophotometer, respectively. Polymerase chain reaction (PCR) in volume 15 µlit for each sample composed of PCR Master Kit (1X), 10pmol primer, 50 ngr DNA and ddH_2O was performed by thermo cycler (Tech Model TC-5000). Five used primers belonged to UBC Company were purchased from Sinaclone-Tehran (Table 1). The PCR program started with an initial phase of 3 min at 95°C followed by 35 cycles of 30s at 95°C, 30s at 30-40°C and 2 min at 72°C; finally elongation for 10 min at 72°C.

PCR products were run on 1.5% electrophoresis gel with a 1X TBE buffer system at 70V by horizontal electrophoresis (Padideh Nogen- Mashhad) along with 100 bp ladder for about two hours and then stained with ethidium bromide. The DNA profiles were scored visually as 0 (no band) and 1 (presence of band) for each primer. The numbers of bands, polymorphism percent of each primer, Polymorphism Information Content or $PIC=(\sum(1-p_i^2-q_i^2)/Np)$ and Marker Index or $MI = PIC.N_p.\beta$ were calculated by EXCEL where p = band frequency and q = no-band frequency, Np is the number of polymorphic bands and β is the polymorphism percentage of each primer (Dalla Rizza *et al*., 2007 and Powell *et al*., 1996). Jaccard similarity matrix, cluster analysis by UPGMA and Mantel test for Cophenetic Correlation Coefficient (CCC) were calculated by NTSYS-pc, 2.02e and finally Analysis of Molecular Variance (AMOVA) was performed by GenAlEx 6.501.

Table 1: ISSR primers, their sequences, total number of produced bands, polymorph bands number, polymorphism percentage, Polymorphism Information Content (PIC) and Marker Index (MI).

Primer code	Primer sequence	Total bands no.	Polymorph bands no.	Polymorphism percentage (%)	PIC	MI
UBC-823	$(TC)_8C$	12	10	83.3	0.454	3.78
UBC-841	$(GA)_8YC$	16	11	68.8	0.458	3.46
UBC-846	$(CA)_8RT$	12	11	91.7	0.450	4.58
UBC-855	$(AC)_8YT$	10	8	80.0	0.468	3.13
UBC-873	(GACA)4	15	10	66.7	0.470	3.10
Average		13	10	78.1	0.460	3.61

RESULTS AND DISCUSSION

Generated ISSR bands on agarose gel of nine ecotypes from three *Salvia* L. species (Fig. 1) showed that the most number of bands was belonged to UBC-841 primer (16 bands), the lowest number of bands was for UBC-855 (10 bands) and the average bands number was 13. From 65 scored DNA bands, 50 bands (76.9%) showed polymorphism that was averagely 10 bands for each primer. Peng *et al*., 2014 reported 14.6 bands and 93.2% polymorphism in 59 different accessions of *S. miltiorrhiza* were reported by using ISSR marker. The lowest and highest PIC values were 0.45 and 0.47 for UBC-846 and UBC-873, respectively. The highest amount of MI belonged to UBC-846 (4.58) that showed high ability of this primer for generating more polymorphism (91.7%) despite of its low PIC. On the other hand the primer UBC-873 showed high value of PIC while it had low MI (3.1) which caused by its low polymorphism bands (66.7%). The average values of PIC and MI for all primers were 0.46 and 3.61, respectively (Table 1). The results of cluster analysis with Jaccard similarity coefficient and UPGMA algorithm revealed that the ecotypes of each species were placed in a separate group. So ISSR marker in this study could distinguish species from each other (Fig. 2). Calculated cophenetic coefficient (0.99) by Mantel test verified suitable fitness of similarity matrix on dendrogram, too. Zhang *et al*., 2013 showed that the dendrograms of 55 *S. miltiorrhiza* germplasms based on ISSR markers has not indicated clear pattern of clustering according to the collection locations. The genetic variation and association within and between the eight species of *Ocimum* genus have investigated by RAPD technique which showed high polymorphism (92.3%) of bands indicating high genetic variation between the species (Sairkar *et al*., 2012).

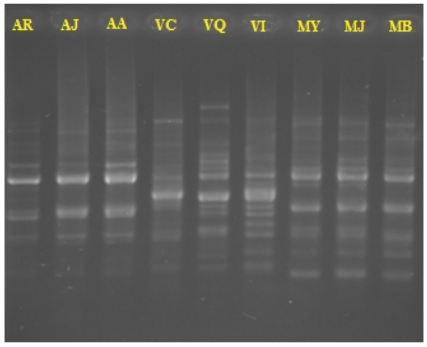

Fig. 1. Electrophoresis gel of studied ecotypes from DNA fragments produced by UBC-873.

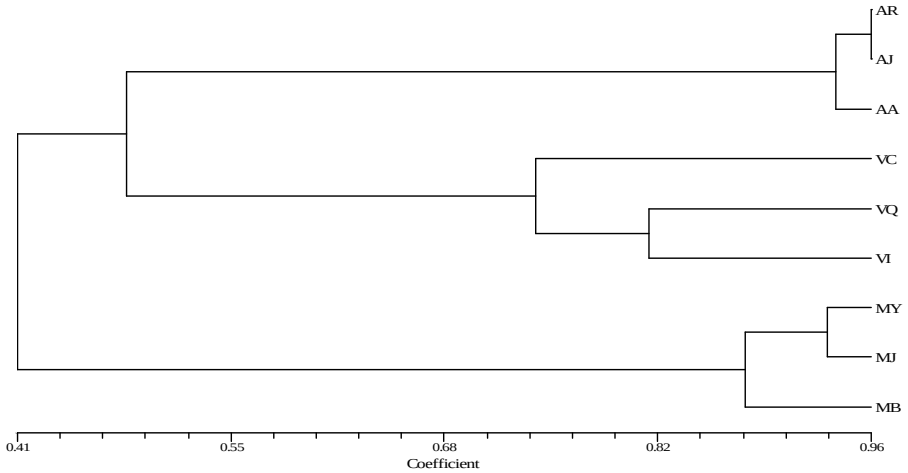

Fig. 2. UPGMA cluster dendrogram based on Jaccard similarity coefficient for studied *Salvia* L. ecotypes (AR, AJ and AA are the ecotypes of Roudbar, Chalous and Ardabil from *S. aethiopsis*, respectively*;* VC, VQ and VI are the ecotypes of Chalous, Qazvin and Ardabil from *S. virgata*, respectively and also MY, MJ and MB are the ecotypes of Yazd, Jahrom and Boushehr from *S. macrosiphon*, respectively).

Table 2: Analysis of molecular variance (AMOVA) between and within *Salvia* L. species by ISSR marker.

S. O. V	df	MS	Variance components	Changing (%)	Probability level
Between species	2	43.1	13.4	82	≤0.01
Within species	6	2.9	2.9	18	≤0.01
Total	8	-	16.3	100	-

Analysis of molecular variance (AMOVA) on three species showed that the diversity within species (18%) was lower than the variation between species (82%) (Table 2). This result stated high ability of ISSR technique in species separation, but for more detailed investigations it is required the techniques with high genetic differentiation. Low variation within *Salvia* ecotypes was reported by Wang *et al.*, 2011, so that ecotypes of S .miltiorrhiza were separated as a single group. The genetic variation of ten endemic and migrated populations of *S. officinalis* studied by RAPD marker could divide them in three separated groups (Echeverrigaray and Agostini 2006). Also they reported that low genetic variation within endemic species could limit gene pool for breeding program and it is better to use immigrant ecotypes as a source of diversification. The study of Peng *et al*., 2014 on *S. miltiorrhiza* populations revealed a considerable level of genetic differentiation among wild and cultivated ones.

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