UDC 575.630
https://doi.org/10.2298/GENSR2102559Y
Original scientific article

GENETIC DIVERSITY AND RELATIONSHIPS AMONG *SALVIA* SPECIES BY ISSR MARKERS

Zhao YANPENG[1], Wang HONGMEI[2*], Liang WEI[3], Majid KHAYATNEZHAD[4] , FAISAL[5]

[1]Department of Fundamental Courses, Shandong University of Science and Technology, Taian, Shandong, China
[2]Dept. of Physical Education, University of Science and Technology Liaoning, Anshan, China
[3]Institute of Physical Education, Qingdao University of Science and Technology, Qingdao Shandong 266061, China
[4]Department of Environmental Sciences and Engineering, Ardabil Branch, Islamic Azad University, Ardabil, Iran
[5] Institute of Plant Sciences, University of Sindh, Jamshoro, Pakistan.

Yanpeng Z., W. Hongmei, L. Wei, M. Khayatnezhad, Faisal (2021). *Genetic diversity and relationships among Salvia species by ISSR markers*. - Genetika, Vol 53, No.2, 559-574.
Species identification is fundamentally important within the fields of conservation, biology, biogeography and ecology. *Salvia* species are herbaceous, biennial or annual, strongly aromatic. Inter-Simple sequence repeats (ISSR) molecular markers were used for evaluate genetic diversity and relationship analysis of 30 *Salvia* species. Ten selected ISSR primers amplified 116 loci, respectively, of which all were polymorphic. The obtained average polymorphism information content 0.39, average band informativeness 10.5 and the marker index 3.1 revealed high genetic diversity prevailing among *Salvia* accessions. The dendrogram was constructed based on ISSR separated the individuals into sub-clusters in accordance with their species. Our results indicated that ISSR markers can be used as a reliable and informative technique for evaluation of genetic diversity and relationships among *Salvia* species. The objectives of present study are: 1) can ISSR markers identify *Salvia* species, 2) what is the genetic of these taxa in Iran, and 3) to investigate the species inter-relationship?
Key words: ISSR, Morphology, Species Identification, STRUCTURE

INTRODUCTION

Species delimitation is important in different biological disciplines, like ecology, biogeography, and plant conservation (MAYR, 1982; WIENS, 2007). Species delimitation is done by tree-based and non-tree-based approaches (SITES and MARSHALL, 2003; ZHANG *et al*., 2020).

Corresponding author: Wang Hongmei, Dept. of Physical Education, University of Science and Technology Liaoning, Anshan, China, E-mail: masxiongg@163.com

In the first method, species form distinguishing clades (phylogenetic species concept), whereas in non-tree-based method, the species are recognized on the basis of gene flow assessments (biological species concept; PÉREZ-LOSADA *et al.*, 2005; MIAO *et al.*, 2019; CONGFEN *et al.*, 2021; HAN *et al*., 2021).

WIENS and PENKROT (2002), mentioned to use morphological data, DNA data and character data for species delimitation while, KNOWLES and CARSTENS (2007) applied how molecular data (i.e., gene trees from DNA sequence data) can be used in species delimitation. The latter authors used coalescent simulations to test the species limits and incorporated data from multiple loci. They indicated the importance of population genetics in species delimitation. Similarly, MEDRANO *et al.* (2014), applied population genetics methods to the species delimitation problem in *Narcissus* Linnaeus (1753: 289) (Amaryllidaceae J.St.-Hil. nom. cons.) by the help of amplified fragment length polymorphism (AFLP) molecular markers.

Salvia L. is known as the largest genus in Lamiaceae (Mentheae-Salviinae) with approximately 1000 species diversified in three regions of the world: Central and South America (500 spp.), Western Asia (200 spp.) and Eastern Asia (100 species) (WALKER *et al.* 2004; DREW *et al.* 2017; WILL and CLAEN-BOCKHOFF, 2017).). Iran included 19 endemic species out of 61 is regarded as one of the important regions for *Salvia* diversity in Southwest Asia (JAMZAD, 2012; JIANG *et al*., 2021; ZHANG *et al*., 2016; WANG *et al.*, 2020). The genus name 'Salvia' is derived from Latin 'Salvio' which means to save, or to recover (WANG *et al*., 2011). *Salvia* is one of the most appreciated herbs for its essential oil richness and biologically active compounds (ERBANO *et al.*, 2015). Using of the genus *Salvia* has been widely increased in the industries such as pharmaceutical because it has pharmacological specifications including, anti-inflammatory, antiplatelet effects (ERBANO *et al.*, 2015). *Salvia* has also been used to treat a number of different diseases such as acquired immunodeficiency syndrome (AIDS), diabetes, liver malfunction and Alzheimer˝s disease (SEPEHRY JAVAN *et al.*, 2012). They also have economical value in the industry of perfumery and cosmetics, and are used as spices and flavoring agents (WANG *et al.*, 2011). Plant genetic diversity is an important factor for their domestication and breeding. Accordingly, some researchers have tried to assess this variability by ISSR and RAPD techniques in different *Salvia* species (SONG *et al*., 2010; WANG *et al*., 2011; SEPEHRY JAVAN *et al*., 2012; ZHANG *et al.*, 2013; PENG *et al*., 2014; ERBANO *et al*., 2015). 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). Genetic variability was also investigated in a genus of Lamiacea family as *Ocimum*, with the application of these markers (CHEN *et al*., 2013; PATEL *et al*., 2014). Germplasm variation detected by ISSRs has been reported in the genus *Stachys*, due to the rich genetic resources of the collection regions; which are important in conservation purposes (KHARAZIAN *et al*., 2015). ISSR molecular markers have employed to show polymorphisms and distinguish *Salvia miltiorrhiza* germplasms by integrating with phenotypic characteristics (ZHANG *et al.*, 2013). ISSR is a simple and efficient marker system for identification of genetic diversity for plant germplasm collection (PENG *et al*., 2014).

Molecular markers provide a powerful tool for studying the genetic diversity. Among advanced genetic markers, Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) markers have been widely used for diversity analyses (PHARMAWATI *et*

al., 2004). RAPD technique is quick, easy and requires no prior sequence information. The technique detects nucleotide sequence polymorphism using a single primer of arbitrary nucleotide sequence (MORENO *et al.*, 1998). ISSR marker involves PCR amplification of DNA by a single 16-18 bp. long primer composed of a repeated sequence anchored at the 3' or 5' end of 2-4 arbitrary nucleotides. The technique is rapid, simple, inexpensive and more reproducible than RAPD (ESFANDANI-BOZCHALOYI *et al.*, 2018a; 2018b; 2018c; 2018d).

The present research was undertaken with the aims of evaluating the genetic diversity of the 30 species of *Salvia*. We try to answer the following questions: 1) Is there infra and interspecific genetic diversity among studied species? 2) Is genetic distance among these species correlated with their geographical distance? 3) What is the genetic structure of populations and taxa? 4) Is there any gene exchange between *Salvia* species in Iran?

MATERIALS AND METHODS

Plant materials

A total of 145 individuals were sampled representing 30 geographical populations belong 30 *Salvia* species in East Azerbaijan, Lorestan, Kermanshah, Guilan, Mazandaran, Golestan, Yazd, Esfahan, Tehran, Arak, Hamadan, Kurdistan, Ilam, Bandar Abbas, Ghazvin, Khorasan and Ardabil Provinces of Iran during July-Agust 2018-2019 (Table 1). For morphometric and ISSR analysis we used 145 plant accessions (one to twelve samples from each populations) belonging to 30 different populations with different eco-geographic characteristics were sampled and stored in -20 till further use. More information about geographical distribution of accessions are in Table 1 and Fig. 1.

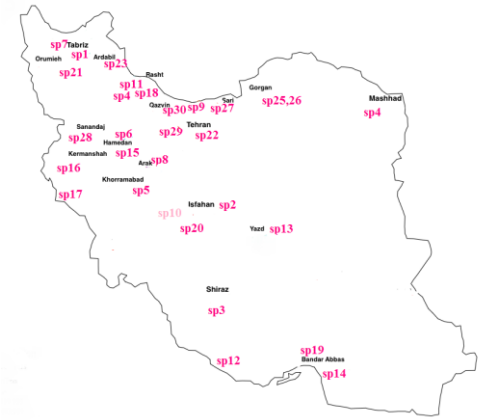

Fig. 1. Map of Iran shows the collection sites and provinces where *Salvia* species were obtained for this study; sp1= *Salvia aristata;* sp2= *S. eremophila;* sp3= *S. santolinifolia*; sp4= *S. tebesana;* sp5= *S. bracteata* ; sp 6= *S. suffruticosa*; sp7= *S. dracocephaloides*; sp8= *S. hydrangea;* sp9= *S. multicaulis*; sp10: *S. syriaca*; sp11: *S. viridis;* sp12= *S. mirzayanii*; sp13= *S. macrosiphon;* sp14= *S. sharifii;* sp15= *S. reuterana;* sp16= *S. palaestina;* sp17= *S. sclareopsis;* sp18= *S. spinose;* sp19= *S. compressa;* sp20= *S. sclarea;* sp21= *S. aethiopis;* sp22= *S. microstegia;* sp23= *S. xanthocheila;* sp24= *S. limbata*; sp25= *S. chloroleuca*; sp26= *S. virgate;* sp27= *S. nemorosa;* sp28= *S. urmiensis*; sp29= *S. oligphylla;* sp30= *S. verticillata*

Table 1. Voucher details of Salvia species and relative genera examined in this study from Iran.

No	Sp.	Locality	Latitude	Longitude	Altitude (m)
Sp1	*Salvia aristata* Aucher ex Benth.	East Azerbaijan, kaleybar, Shojabad	38 ˚ 52'37"	47 ˚ 23' 92"	1144
Sp2	*S. eremophila* Boiss.	Esfahan, Ghameshlou, Sanjab	32°50′03″	51°24′28″	1990
Sp3	*S. santolinifolia* Boiss.	Fars, Jahrom	29◦20′07″	51° 52′08″	1610
Sp4	*S. tebesana* Bunge	Khorasan, Tabas	38 ˚ 52'373	47 ˚ 23' 92"	
Sp5	*S. bracteata* Banks & Sol	Lorestan, Oshtorankuh, above Tihun village	33° 57′12″	47° 57′32″	2500
Sp6	*S. suffruticosa* Montb. & Aucher	Hamedan, Nahavand	34 ˚ 52'373	48 ˚ 23' 92"	2200
Sp7	*S. dracocephaloides* Boiss.	East Azerbaijan, kaleybar, Cheshme Ali Akbar	38 ˚ 52'373	47 ˚ 23' 92"	1144
Sp8	*S. hydrangea* DC. ex Benth.	Arak, Komayjan, Pass of Chehregan village, the margin road	35°50′03″	51°24′28″	1700
Sp9	*S. multicaulis* Vahl.	Mazandaran, Haraz road, Emam Zad-e-Hashem	36°14′14″	51°18′07″	1807
Sp10	*S. syriaca* L.	Esfahan, Fereydunshahr	32◦36′93″	51°27′90″	2500
Sp11	*S. viridis* L.	Guilan, Sangar, Road sid	37°07′02″	49°44′32″	48
Sp12	*S. mirzayanii* Rech. f. & Esfand.	Boushehr, Dashtestan	28◦57′22″	51°28′31″	430
Sp13	*S. macrosiphon* Boiss.	Yazd, Khatam	30◦07′24″	53° 59′06″	2178
Sp14	*S. sharifii* Rech. f. & Esfand.	Bandar Abbas, Hormozgan	28◦57′22″	51°28′31″	288
Sp15	*S. reuterana* Boiss.	Hamedan, Alvand	34◦46′10″	48°30′00″	1870
Sp16	*S. palaestina* Benth.	Kermanshah, Islamabad	35° 37′77″	46° 20′25″	1888
Sp17	*S. sclareopsis* Bornm. ex Hedge	Ilam, Ilam	33°47′60″	46 °07′58″	1250
Sp18	*S. spinose* L.	Guilan, Lahijan	37°07′02″	49°44′32″	48
Sp19	*S. compressa* Vent.	Bandar Abbas, Hormozgan	28◦57′22″	51°28′31″	288
Sp20	*S. sclarea* L.	Esfahan:, Ghameshlou, Sanjab	32◦36′93″	51°27′90″	2500
Sp21	*S. aethiopis* L.	Azerbaijan, 78 km from Mianeh to Khalkhl.	37◦38_53	48 ◦ 36_11	1500
Sp22	*S. microstegia* Boiss. & Bal.	Tehran, Darband	35◦36′93″	51°27′90″	1700
Sp23	*S. xanthocheila* Boiss. ex Benth.	Ardabil, Khalkhal	37◦38_53	48 ◦ 36_11	1958
Sp24	*S. limbata* C. A. Mey.	Guilan,Gole rodbar, Road sid	37 ˚ 09' 45"	49 ˚ 55' 39 "	15
Sp25	*S. chloroleuca* Rech. f. & Aell.	Golestan, Ramian	37 ˚ 09' 45"	55 ˚ 55' 39 "	1320
Sp26	*S. virgate* Jacq.	Golestan, Ramian	37 ˚ 09' 45"	55 ˚ 55' 39 "	1320
Sp27	*S. nemorosa* L.	Mazandaran, Chalos	36°14′14″	51°18′07″	1807
Sp28	*S. urmiensis* Bunge	Kurdistan, Sanandaj	37 ˚ 09' 45"	55 ˚ 55' 39 "	1320
Sp29	*S. oligphylla* Aucher ex Benth.	Ghazvin to Hamedan just after Avaj	35◦36′93″	51°27′90″	2100
Sp30	*S. verticillata* L.	Mazandaran Jadeh Chalous	36°14′14″	51°18′07″	1807

Morphological studies

1-12 samples from each species were used for Morphometry. In total 22 morphological (9 qualitative, 13 quantitative) characters were studied. Data obtained were standardized (Mean= 0, variance = 1) and used to estimate Euclidean distance for clustering and ordination analyses (PODANI, 2000). Morphological characters studied are: corolla shape, bract shape, seed color, seed shape, bract color, corolla latex, leaf surface, calyx shape, basal leaf shape, pedicel length, calyx length, bract length, filament length, anther length, corolla length, nut length, nut width, basal leaf length, basal leaf width, corolla color, stem leaf length and stem leaf width.

DNA extraction and ISSR assay

Fresh leaves were used randomly from one to twelve plants in each of the studied populations. These were dried by silica gel powder. CTAB activated charcoal protocol was used to extract genomic DNA (ESFANDANI-BOZCHALOYI *et al.*, 2019). For the ISSR analysis, 22 primers from UBC (University of British Columbia) series were tested for DNA amplification. Ten primers were chosen for ISSR analysis of genetic variability, based on band reproducibly (Table 2). PCR reactions were carried in a 25µl volume containing 10 mM Tris-HCl buffer at pH 8; 50 mM KCl; 1.5 mM $MgCl_2$; 0.2 mM of each dNTP (Bioron, Germany); 0.2 µM of a single primer; 20 ng genomic DNA and 3 U of *Taq* DNA polymerase (Bioron, Germany). The amplifications, reactions were performed in Techne thermocycler (Germany) with the following program: 5 min initial denaturation step 95°C, followed by 37 cycles of 1 min at 95°C; 1 min at 50-56°C and 1 min at 72°C. The reaction was completed by final extension step of 5-10 min at 72°C. The amplification products were observed by running on 1% agarose gel, followed by the ethidium bromide staining. The fragment size was estimated by using a 100 bp molecular size ladder (Fermentas, Germany).

Table 2. Details about the banding pattern revealed by ISSR primers.

Primer name	Primer sequence (5'-3')	TNB	NPB	PPB	PIC	PI	EMR	MI
ISSR-1	DBDACACACACACACACA	10	10	100.00%	0.38	5.86	7.55	2.45
ISSR-2	GGATGGATGGATGGAT	11	10	91.00%	0.48	4.91	7.43	2.85
ISSR-3	GACAGACAGACAGACA	12	10	83.00%	0.36	5.34	8.55	3.44
ISSR-4	AGAGAGAGAGAGAGAGYT	10	10	100.00%	0.43	4.88	8.56	3.65
ISSR-5	ACACACACACACACACC	13	13	100.00%	0.25	5.23	7.23	2.47
ISSR-6	GAGAGAGAGAGAGAGARC	11	9	91.00%	0.35	4.66	7.56	2.67
ISSR-7	CTCTCTCTCTCTCTCTG	13	10	77.00%	0.44	3.21	9.60	4.55
ISSR-8	CACACACACACACACAG	13	11	92.00%	0.32	4.32	9.55	4.45
ISSR-9	GTGTGTGTGTGTGTGTYG	12	10	83.00%	0.45	4.56	7.34	2.11
ISSR-10	CACACACACACACACARG	11	10	91.00%	0.47	4.25	7.11	2.87
	Average	11.6	10.5	90.00%	0.39	4.72	7.66	3.1

TNB - the number of total bands, NPB: the number of polymorphic bands, PPB (%): the percentage of polymorphic bands, PI: polymorphism index, EMR, effective multiplex ratio; MI, marker index; PIC, polymorphism information content for each of CBDP primers

Data analyses

Morphological studies

Morphological characters were first standardized (Mean = 0, Variance = 1) and used to establish Euclidean distance among pairs of taxa (PODANI, 2000). For grouping of the plant specimens, The UPGMA (Unweighted paired group using average), were used (PODANI, 2000). ANOVA (Analysis of variance) were performed to show morphological difference among the populations while, PCA (Principal components analysis) biplot was used to identify the most variable morphological characters among the studied populations (PODANI, 2000). PAST version 2.17 (HAMMER *et al.*, 2012) was used for multivariate statistical analyses of morphological data.

Molecular analyses

The ISSR profiles obtained for each samples were scored as binary characters. Discriminatory ability of the used primers was evaluated by means of two parameters, polymorphism information content (PIC) and marker index (MI) to characterize the capacity of each primer to detect polymorphic loci among the genotypes (POWELL *et al.*, 1996). MI is calculated for each primer as MI = PIC × EMR, where EMR is the product of the number of polymorphic loci per primer (*n*) and the fraction of polymorphic fragments (β) (HEIKRUJAM *et al.*, 2015). The number of polymorphic bands (NPB) and the effective multiplex ratio (EMR) were calculated for each primer. Parameter like Nei's gene diversity (H), Shannon information index (I), number of effective alleles, and percentage of polymorphism (P% =number of polymorphic loci/number of total loci) were determined (WEISING *et al.*, 2005; FREELAND *et al.*, 2011).

Shannon's index was calculated by the formula: H' = -Σpiln pi. Rp is defined per primer as: Rp = ∑ Ib, were "Ib" is the band informativeness, that takes the values of 1-(2x [0.5-p]), being "p" the proportion of each genotype containing the band. The percentage of polymorphic loci, the mean loci by accession and by population, UHe, H' and PCA were calculated by GenAlEx 6.4 software (PEAKALL and SMOUSE, 2006). Nei's genetic distance among populations was used for Neighbor Joining (NJ) clustering and Neighbor-Net networking (FREELAND *et al.*, 2011; HUSON and BRYANT, 2006). Mantel test checked the correlation between geographical and genetic distances of the studied populations (PODANI, 2000). These analyses were done by PAST ver. 2.17 (HAMMER *et al.*, 2012), DARwin ver. 5 (2012) software. AMOVA (Analysis of molecular variance) test (with 1000 permutations) as implemented in GenAlex 6.4 (PEAKALL and SMOUSE, 2006) were used to show genetic difference of the populations. Gene flow was determined by (i) Calculating Nm an estimate of gene flow from Gst by PopGene ver. 1.32 (1997) as: Nm = 0.5(1 - Gst)/Gst. This approach considers the equal amount of gene flow among all populations.

RESULTS

Species identification and inter-relationship

Morphometry

ANOVA showed significant differences ($P < 0.01$) in quantitative morphological characters among the species studied. In order to determine the most variable characters among

the taxa studied, PCA analysis has been performed. It revealed that the first three factors comprised over 73% of the total variation. In the first PCA axis with 52% of total variation, such characters as calyx length, bract length, seed shape, calyx shape, and basal leaf shape have shown the highest correlation (>0.7), The corolla length, filament length, nut width, basal leaf length, seed color, and leaf surface were characters influencing PCA axis 2 and 3 respectively. Different clustering and ordination methods produced similar results therefore, MDS plot of morphological characters are presented here (Figs 2). In general, plant samples of each species were grouped together and formed separate groups. These results show that both quantitative and qualitative morphological characters separated the studied species into distinct groups. In the studied specimens we did not encounter intermediate forms.

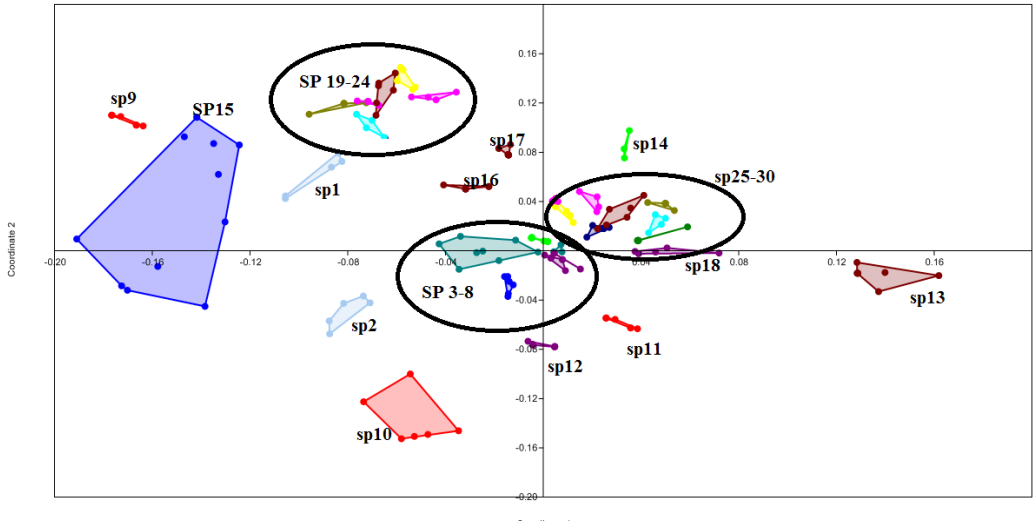

Figure 2. MDS plots of morphological characters revealing species delimitation in the *Salvia*
sp1= *Salvia aristata;* sp2= *S. eremophila;* sp3= *S. santolinifolia*; sp4= *S. tebesana;* sp5= *S. bracteata* ; sp 6= *S. suffruticosa*; sp7= *S. dracocephaloides*; sp8= *S. hydrangea;* sp9= *S. multicaulis*; sp10: *S. syriaca*; sp11: *S. viridis;* sp12= *S. mirzayanii*; sp13= *S. macrosiphon;* sp14= *S. sharifii;* sp15= *S. reuterana;* sp16= *S. palaestina;* sp17= *S. sclareopsis;* sp18= *S. spinose;* sp19= *S. compressa;* sp20= *S. sclarea;* sp21= *S. aethiopis;* sp22= *S. microstegia;* sp23= *S. xanthocheila;* sp24= *S. limbata*; sp25= *S. chloroleuca*; sp26= *S. virgate;* sp27= *S. nemorosa;* sp28= *S. urmiensis*; sp29= *S. oligphylla;* sp30= *S. verticillata*

Species Identification and Genetic Diversity

Ten ISSR primers were screened to study genetic relationships among *Salvia* species; all the primers produced reproducible polymorphic bands in all 30 *Salvia* species. An image of the ISSR amplification generated by ISSR-5 primer is shown in Figure 3. A total of 105 amplified polymorphic bands were generated across 30 *Salvia* species. The size of the amplified

fragments ranged from 100 to 2500 bp. The highest and lowest number of polymorphic bands was 13 for ISSR-5 and 9 for ISSR-6, on an average of 10.5 polymorphic bands per primer. The PIC of the 10 ISSR primers ranged from 0.32 (ISSR-8) to 0.48 (ISSR-2) with an average of 0.39 per primer. MI of the primers ranged from 2.11 (ISSR-9) to 4.55 (ISSR-7) with an average of 3.1 per primer. EMR of the ISSR primers ranged from 7.11 (ISSR-10) to 9.60 (ISSR-7) with an average of 7.66 per primer (Table 2). The primers with the high EMR values were considered to be more informative in distinguishing the genotypes.

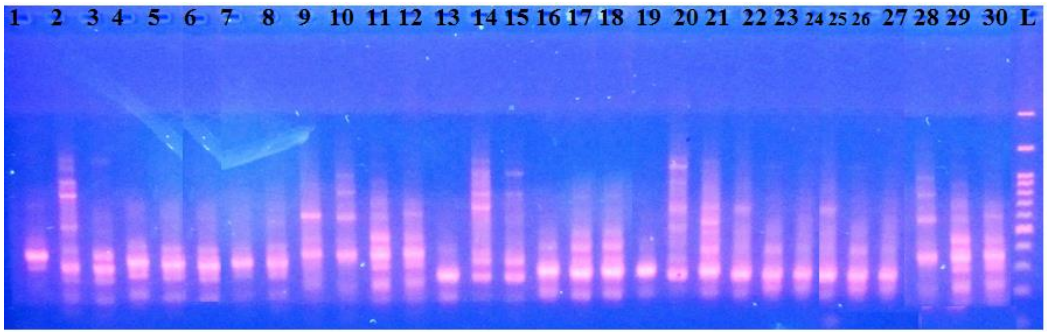

Fig. 3. Electrophoresis gel of studied ecotypes from DNA fragments produced by ISSR-6.

1= *Salvia aristata;* 2= *S. eremophila;* 3= *S. santolinifolia*; 4= *S. tebesana;* 5= *S. bracteata* ; 6= *S. suffruticosa*; 7= *S. dracocephaloides*; 8= *S. hydrangea;* 9= *S. multicaulis*; 10: *S. syriaca*; 11: *S. viridis;* 12= *S. mirzayanii*; 13= *S. macrosiphon;* 14= *S. sharifii;* 15= *S. reuterana;* 16= *S. palaestina;* 17= *S. sclareopsis;* 18= *S. spinose;* 19= *S. compressa;* 20= *S. sclarea;* 21= *S. aethiopis;* 22= *S. microstegia;* 23= *S. xanthocheila;* 24= *S. limbata*; 25= *S. chloroleuca*; 26= *S. virgate;* 27= *S. nemorosa;* 28= *S. urmiensis*; 29= *S. oligphylla;* 30= *S. verticillata;*L = Ladder 100 bp, Arrows are representative of polymorphic bands

The genetic parameters were calculated for all the 30 *Salvia* species amplified with ISSR primers (Table 3). Unbiased expected heterozygosity (*H*) ranged from 0.1 (*S. sclareopsis*) to 0.3 (*S. bracteata*), with a mean of 0.23. A similar pattern was observed for Shannon's information index (*I)*, with the highest value of 0.44 observed in *S. bracteata* and the lowest value of 0.1 observed in *S. sclareopsis* with a mean of 0.22. The observed number of alleles (*N*a) ranged from 0.16 in *S. spinose to* 1.255 in *S. macrosiphon*. The effective number of alleles (*N*e) ranged from 1 (*S. tebesana*) to 1.35 (*S. chloroleuca*).

AMOVA test showed significant genetic difference (P = 0.01) among studied species. It revealed that 79% of total variation was among species and 21% was within species (Table 4) Moreover, genetic differentiation of these species was demonstrated by significant Nei's GST (0.41, P = 0.01) and D_est values (0.169, P = 0.01). These results revealed a higher distribution of genetic diversity among *Salvia* species compared to within species.

Table 3. Genetic diversity parameters in the studied Salvia species.

SP	N	Na	Ne	I	He	UHe	%P
S. aristata	6.000	0.892	1.268	0.221	0.151	0.165	37.63%
S. eremophila	6.000	0.344	1.035	0.36	0.23	0.25	46.53%
S. santolinifolia	4.000	0.312	1.031	0.28	0.18	0.21	45.38%
S. tebesana	8.000	0.301	1.00	0.23	0.17	0.12	37.23%
S. bracteata	5.000	0.441	1.038	0.44	0.37	0.30	54.75%
S. suffruticosa	3.000	0.667	1.162	0.14	0.224	0.113	24.73%
S. dracocephaloides	5.000	0.376	1.054	0.23	0.35	0.19	31.83%
S. hydrangea	4.000	0.366	1.043	0.40	0.26	0.20	47.53%
S. multicaulis	5.000	0.269	1.021	0.15	0.18	0.12	32.15%
S. syriaca	8.000	0.548	1.013	0.23	0.12	0.12	39.68%
S. viridis	9.000	0.452	1.089	0.18	0.22	0.25	45.05%
S. mirzayanii	8.000	0.333	1.006	0.344	0.23	0.26	43.23%
S. macrosiphon	12.000	1.255	1.304	0.28	0.184	0.192	35.91%
S. sharifii	5.000	0.258	1.017	0.18	0.15	0.12	34.30%
S. reuterana	6.000	0.258	1.029	0.28	0.18	0.20	42.38%
S. palaestina	5.000	0.462	1.095	0.277	0.25	0.22	45.05%
S. sclareopsis	8.000	0.699	1.167	0.11	0.101	0.107	22.26%
S. spinose	9.000	0.161	1.004	0.122	0.33	0.27	42.15%
S. compressa	6.000	0.355	1.041	0.39	0.25	0.28	47.53%
S. sclarea	10.000	0.441	1.036	0.33	0.22	0.23	47.53%
S. aethiopis	3.000	0.247	1.021	0.15	0.18	0.13	32.15%
S. microstegia	3.000	0.290	1.024	0.23	0.15	0.18	34.30%
S. xanthocheila	9.000	0.452	1.089	0.23	0.32	0.15	35.05%
S. limbata	8.000	0.333	1.006	0.122	0.12	0.22	43.23%
S. chloroleuca	12.000	1.247	1.35	0.271	0.184	0.192	35.91%
S. virgata	5.000	0.258	1.017	0.174	0.11	0.12	34.30%
S. nemorosa	6.000	0.258	1.029	0.231	0.18	0.20	45.38%
S. urmiensis	5.000	0.462	1.095	0.288	0.25	0.22	15.05%
S. oligphylla	8.000	0.699	1.167	0.259	0.122	0.167	32.26%
S. verticillata	8.000	0.699	1.167	0.356	0.133	0.145	36.26%

N = number of samples, Na= number of different alleles; Ne = number of effective alleles, I= Shannon's information index, He = gene diversity, UHe = unbiased gene diversity, P%= percentage of polymorphism, populations.

Table 4. Analysis of molecular variance (AMOVA) of the studied species.

Source	df	SS	MS	Est. Var.	%	*ΦPT*
Among Pops	22	1601.364	72.789	11.154	79%	
Within Pops	122	354.443	2.905	2.905	21%	79%
Total	144	1955.807		14.060	100%	

df: degree of freedom; SS: sum of squared observations; MS: mean of squared observations; EV: estimated variance; *Φ*PT: proportion of the total genetic variance among individuals within an accession, ($P < 0.001$).

Table 5. The matrix of Nei genetic similarity (Gs) estimates using ISSR molecular markers among 30 Salvia species.

	sp1	sp2	sp3	sp4	sp5	sp6	sp7	sp8	sp9	sp10	sp11	sp12	sp13	sp14	sp15	sp16	sp17	sp18	sp19	sp20	sp21	sp22	sp23	sp24	sp25	sp26	sp27	sp28	sp29	sp30	
sp1	1.000																														sp1
sp2	0.757	1.000																													sp2
sp3	0.703	0.738	1.000																												sp3
sp4	0.701	0.758	0.842	1.000																											sp4
sp5	0.675	0.792	0.786	0.816	1.000																										sp5
sp6	0.695	0.721	0.767	0.770	0.793	1.000																									sp6
sp7	0.724	0.774	0.823	0.831	0.836	0.862	1.000																								sp7
sp8	0.667	0.723	0.781	0.832	0.823	0.846	0.928	1.000																							sp8
sp9	0.672	0.716	0.749	0.787	0.766	0.808	0.875	0.951	1.000																						sp9
sp10	0.675	0.602	0.681	0.666	0.683	0.618	0.708	0.704	0.680	1.000																					sp10
sp11	0.730	0.782	0.817	0.794	0.776	0.816	0.884	0.812	0.820	0.721	1.000																				sp11
sp12	0.649	0.659	0.715	0.709	0.660	0.752	0.754	0.703	0.725	0.635	0.839	1.000																			sp12
sp13	0.757	0.747	0.645	0.740	0.771	0.712	0.757	0.717	0.672	0.632	0.725	0.642	1.000																		sp13
sp14	0.798	0.708	0.745	0.692	0.696	0.737	0.759	0.709	0.680	0.667	0.762	0.728	0.684	1.000																	sp14
sp15	0.694	0.742	0.839	0.761	0.763	0.807	0.859	0.785	0.775	0.666	0.869	0.796	0.676	0.722	1.000																sp15
sp16	0.783	0.793	0.759	0.791	0.775	0.782	0.872	0.792	0.773	0.649	0.840	0.709	0.770	0.754	0.770	1.000															sp16
sp17	0.787	0.759	0.641	0.683	0.676	0.702	0.740	0.671	0.650	0.617	0.746	0.676	0.699	0.756	0.735	0.778	1.000														sp17
sp18	0.752	0.860	0.767	0.725	0.769	0.814	0.802	0.757	0.716	0.599	0.835	0.758	0.746	0.753	0.795	0.799	0.756	1.000													sp18
sp19	0.752	0.774	0.784	0.758	0.785	0.790	0.817	0.784	0.770	0.641	0.825	0.722	0.757	0.779	0.798	0.834	0.750	0.799	1.000												sp19
sp20	0.676	0.767	0.827	0.825	0.795	0.754	0.800	0.751	0.774	0.732	0.846	0.755	0.649	0.675	0.808	0.768	0.675	0.727	0.755	1.000											sp20
sp21	0.786	0.733	0.701	0.731	0.665	0.681	0.723	0.683	0.659	0.679	0.714	0.636	0.729	0.691	0.665	0.720	0.681	0.746	0.684	0.711	1.000										sp21
sp22	0.709	0.794	0.764	0.713	0.791	0.756	0.844	0.804	0.793	0.695	0.858	0.703	0.746	0.734	0.799	0.829	0.733	0.599	0.848	0.774	0.712	1.000									sp22
sp23	0.721	0.794	0.754	0.717	0.795	0.751	0.826	0.786	0.772	0.686	0.836	0.681	0.745	0.744	0.778	0.816	0.740	0.785	0.846	0.757	0.707	0.980	1.000								sp23
sp24	0.755	0.759	0.636	0.635	0.668	0.651	0.691	0.632	0.615	0.602	0.711	0.624	0.657	0.735	0.706	0.719	0.953	0.741	0.690	0.657	0.645	0.726	0.735	1.000							sp24
sp25	0.771	0.873	0.773	0.726	0.773	0.809	0.802	0.755	0.730	0.614	0.843	0.759	0.739	0.750	0.797	0.812	0.774	0.990	0.797	0.741	0.755	0.799	0.786	0.757	1.000						sp25
sp26	0.752	0.774	0.784	0.758	0.785	0.790	0.817	0.784	0.770	0.641	0.825	0.722	0.757	0.779	0.798	0.834	0.750	0.799	1.000	0.755	0.684	0.848	0.846	0.690	0.797	1.000					sp26
sp27	0.677	0.783	0.844	0.839	0.806	0.755	0.812	0.761	0.762	0.736	0.860	0.759	0.661	0.689	0.825	0.778	0.691	0.744	0.768	0.992	0.715	0.787	0.770	0.673	0.755	0.768	1.000				sp27
sp28	0.787	0.744	0.701	0.720	0.655	0.669	0.712	0.672	0.670	0.669	0.726	0.647	0.719	0.701	0.676	0.510	0.688	0.757	0.690	0.700	0.989	0.723	0.714	0.656	0.767	0.690	0.704	1.000			sp28
sp29	0.709	0.794	0.764	0.713	0.791	0.756	0.844	0.804	0.793	0.695	0.858	0.703	0.746	0.734	0.799	0.829	0.733	0.800	0.848	0.774	0.712	0.765	0.654	0.726	0.799	0.848	0.787	0.723	1.000		sp29
sp30	0.721	0.794	0.754	0.717	0.795	0.751	0.826	0.786	0.772	0.686	0.836	0.681	0.745	0.744	0.778	0.816	0.740	0.785	0.846	0.757	0.707	0.980	0.876	0.735	0.786	0.846	0.770	0.714	0.654	1.000	sp30

sp1= Salvia aristata; sp2= S. eremophila; sp3= S. santolinifolia; sp4= S. tebesana; sp5= S. bracteata ; sp 6= S. suffruticosa; sp7= S. dracocephaloides; sp8= S. hydrangea; sp9= S. multicaulis; sp10: S. syriaca; sp11: S. viridis; sp12= S. mirzayanii; sp13= S. macrosiphon; sp14= S. sharifii; sp15= S. reuterana; sp16= S. palaestina; sp17= S. sclareopsis; sp18= S. spinose; sp19= S. compressa; sp20= S. sclarea; sp21= S. aethiopis; sp22= S. microstegia; sp23= S. xanthocheila; sp24= S. limbata; sp25= S. chloroleuca; sp26= S. virgate; sp27= S. nemorosa; sp28= S. urmiensis; sp29= S. oligphylla; sp30= S. verticillata

Two major clusters were formed in NJ tree (Fig. 4). The first major cluster contained two sub-clusters: plants of *Salvia chloroleuca*, *S. nemorosa*, *S. urmiensis* comprised the first sub-cluster. The second sub-cluster was formed by *S. xanthocheila*, *S. limbata*, *S. aethiopis*, *S. sclarea*, *S. virgate*, *S. microstegia* and *S. compressa*. The second major cluster also contained two sub-clusters, *S. reuterana* are separated from the other studied species and join the others with a great distance. This dendrogram showed close genetic affinity between other species. In general, relationships obtained from ISSR data agrees well with species relationship obtained from morphological. This is in agreement with AMOVA and genetic diversity parameters presented before. The species are genetically well differentiated from each other.

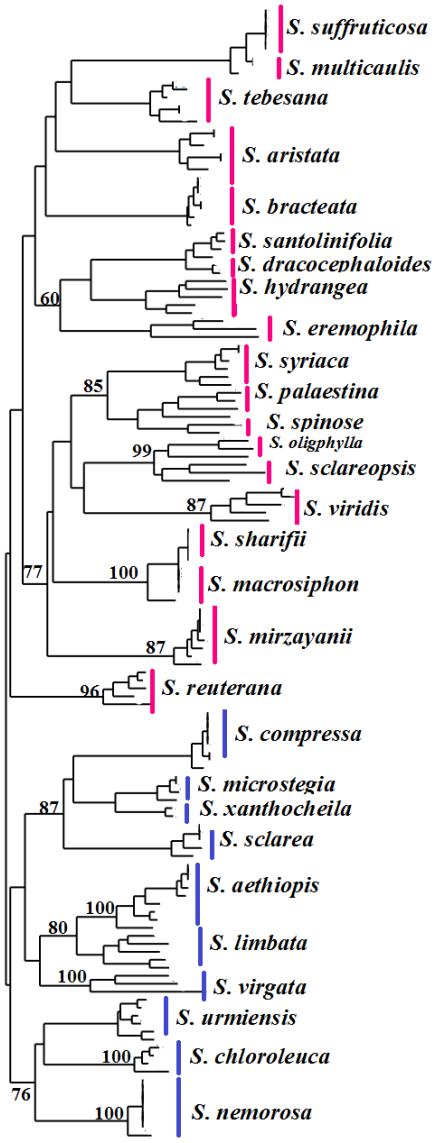

Figure 4. Neighbor joining tree of inter simple sequence repeats data revealing species delimitation in the *Salvia*

These results indicate that ISSR molecular markers can be used in *Salvia* species taxonomy. The Nm analysis by Popgene software also produced mean Nm= 0.23, that is considered very low value of gene flow among the studied species.

Mantel test with 5000 permutations showed a significant correlation (r = 0.18, p=0.0001) between genetic distance and geographical distance, so isolation by distance (IBD) occurred among the *Salvia* species studied.

Nei's genetic identity and the genetic distance determined among the studied species (Table 5). The results showed that the highest degree of genetic similarity (0.98) occurred between *Salvia sclarea* and. *S. nemorosa.* The lowest degree of genetic similarity occurred between *S. palaestina* and *S. urmiensis* (0.51). The low Nm value (0.23) indicates limited gene flow or ancestrally shared alleles between the species studied and indicating high genetic differentiation among and within *Salvia* species.

DISCUSSION

Genetic diversity

The Genetic diversity is of fundamental importance in the continuity of a species as it is used to bring about the necessary adaptation to the cope with changes in the environment (TAMS *et al*., 2005). Degree of genetic variability within a species is highly correlated with its reproductive mode, the higher degree of open pollination/ cross breeding brings about higher level of genetic variability in the studied taxon (WANG *et al.*, 2009; ESFANDANI-BOZCHALOYI *et al.*, 2017a; 2017b; 2017c; 2017d).

Salvia as an important medical herb has medicine applications. The reduction of genetic diversity in the *Salvia* genus through domestication has resulted in the need for collection, conservation, characterization and utilization of its genetic resources. During the present research, a gene targeted molecular markers, i.e. ISSR was adapted to study the relationships and genetic diversity among/within different *Salvia* species collected from different regions of Iran.

PIC and MI characteristics of a primer help in determining its effectiveness in genetic diversity analysis. SIVAPRAKASH *et al.* (2004) suggested that the ability of a marker technique to resolve genetic diversity may be more directly related to the degree of polymorphism. Generally, PIC value between zero to 0.25 imply a very low genetic diversity among genotypes, between 0.25 to 0.50 shows a mid-level of genetic diversity and value ≥0.50 suggests a high level of genetic diversity (TAMS *et al.,* 2005). In this research, the ISSR primers' PIC values ranged from 0.25 to 0.48, with a mean value of 0.39, which indicated a mid-ability of ISSR primers in determining genetic diversity among the *Salvia* species. Comparable but low PIC values have been reported with other markers like RAPD and AFLP in African plantain (UDE *et al.*, 2003), ISSR and RAPD in *Salvia* species (YOUSEFIAZAR-KHANIAN *et al*., 2016), AFLP in wheat (BOHN *et al.*, 1999) and SCoT markers (ETMINAN *et al.*, 2018; POUR-ABOUGHADAREH *et al*., 2017; 2018). In HEIKRUJAM *et al.* (2015), CBDP markers were found to be more effective than SCoT markers with regard to the average PIC which was higher. In our study, the ISSR markers were found to be effective in the estimation of different *Salvia* species genetic diversity with regard to average percentage polymorphism (90%), average PIC value of ISSR markers (0.39), average MI (3.1) and average EMR of ISSR markers (7.66), which were higher than other reported markers on *Salvia* (WANG *et al.*, 2009; SONG *et al.*, 2010; YOUSEFIAZAR-KHANIAN *et al.*, 2016;

ETMINAN *et al*., 2018). However, various marker techniques were found to have different resolution of the genome regions and the number of loci that cover the whole genome for estimating of genetic diversity (SOUFRAMANIEN and GOPALAKRISHNA, 2004). A diverse level of polymorphism in *Salvia* species using ISSR, CoRAP, SRAP, SCoT and RAPD markers had been reported earlier by WANG and ZHANG (2009), SONG *et al*. (2010), YOUSEFIAZAR-KHANIAN *et al*. (2016) and ETMINAN *et al.* (2018). Gene flow is inversely correlated with the gene differentiation but is very important for population evolution, and takes place by pollen and seeds between populations (SONG *et al.*, 2010). In the current study, detected gene flow (*N*m) among *Salvia* species was 0.23, showed low genetic differentiation among *Salvia* species.

As a general rule, insects are the pollinators of *Salvia* in Old World (CLAßEN-BOCKHOFF *et al*., 2004). At the lower elevations, bees and at the higher altitudes insects like flies are the dominate pollinators among bilabiate flowers such as *Salvia* (PELLISSIER *et al*., 2010).

According to MOEIN *et al.* (2019) genetic structure of SRAP marker showed that despite the presence of a limited gene flow, two distinct ecotypes were formed which may be the consequences of reproductive isolation caused by altitude gradient and different niches through parapatric speciation. The heterozygosity (*H*) and Shannon index (*I*) reflect diversity and differentiation among and within the germplasm collections, respectively (QUE *et al.*, 2014), and the higher the indices, the greater the genetic diversity. The magnitude of variability among *N*a, *N*e, *H* and *I* indices using studied ISSR markers demonstrated a high level of genetic diversity among and within *Salvia* ecotypes/ species. In conclusion, the results of this study showed that to evaluate the genetic diversity of the *Salvia* genus, the primers derived from ISSR were more effective than the other molecular markers. Also, *Salvia* ecotypes/species were clearly separated from each other in the dendrogram and MDS, indicating the higher efficiency of ISSR technique in *Salvia* species identification.

Received, March 25th, 2020
Accepted January 18th, 2021

REFERENCES

BOHN, M., H.F., UTZ, A.E., MELCHINGER (1999): Genetic similarities among wheat cultivars determined on the basis of RFLPs, AFLPs and SSRs and their use for predicting progeny variance. Crop Sci., *39*: 228–237.

CHEN, S.Y., T.X., DAI, Y.T., CHANG, S.S., WANG, S.L., OU, W.L., CHUANG, C.Y., CHENG Y.H., L.Y., LIN LIN, H.M., KU (2013): Genetic diversity among *Ocimum* species based on ISSR, RAPD and SRAP markers. Australian J. Crop Sci.,*7*(10):1463-1471.

CONGFEN, Z., L. YINGXIAO, H. LINFENG, S. FENGJUN, Y.WEITANG,,... L.XUEGANG (2021): Effects of Uranium on the Antioxidant Responses of Chinese Oak Silkworm, Antheraea pernyi. Pakistan Journal of Zoology, 1-8.

CLAßEN-BOCKHOFF, R., T., SPECK, E., TWERASER, P., WESTER, S., THIMM, M., REITH (2004): The staminal lever mechanism in Salvia L. (Lamiaceae): a key innovation for adaptive radiation? Org. Divers. Evol., *4*(3): 189–205.

DREW, B.T., J.G., GONZÁLEZ-GALLEGOS, C.L., XIANG (2017): *Salvia* united: the greatest good for the greatest number. Taxon, *66*: 133–145.

ERBANO, M., E., SCHNELL, G., SCHÜHLI, D.O.S., EREIRA, É., SANTOS (2015): Genetic variability and population structure of Salvia lachnostachys: implications for breeding and conservation programs. Int. J. Mol. Sci., *16*(4): 7839–7850.

ETMINAN, A., A., POUR-ABOUGHADAREH, A., NOORIC (2018): Genetic relationships and diversity among wild *Salvia* accessions revealed by ISSR and SCoT markers. Biotechnol. Biotechnol. Equip., *32*: 610–617.

ESFANDANI BOZCHALOYI, S., M., SHEIDAI, M., KESHAVARZI, Z., NOORMOHAMMADI (2017a): Genetic Diversity and Morphological Variability in *Geranium Purpureum* Vill. (Geraniaceae) of Iran. Genetika, *49*: 543-557.

ESFANDANI BOZCHALOYI, S., M., SHEIDAI, M., KESHAVARZI, Z., NOORMOHAMMADI (2017b): Species Delimitation In *Geranium* Sect. *Batrachioidea*: Morphological and Molecular. Act. Bot. Hung., *59*(3–4):319–334.

ESFANDANI BOZCHALOYI, S., M., SHEIDAI, M., KESHAVARZI, Z., NOORMOHAMMADI (2017c): Genetic and morphological diversity in *Geranium dissectum* (Sec. Dissecta, Geraniaceae) populations. Biologia, *72*(10): 1121-1130.

ESFANDANI BOZCHALOYI, S., M., SHEIDAI, M., KESHAVARZI, Z., NOORMOHAMMADI (2017d): Analysis of genetic diversity in *Geranium robertianum* by ISSR markers. Phytologia Balcanica, *23*(2):157–166.

ESFANDANI-BOZCHALOYI, S., M., SHEIDAI, M., KESHAVARZI, Z., NOORMOHAMMADI (2018a): Species Relationship and Population Structure Analysis In *Geranium* Subg. *Robertium* (Picard) Rouy With The Use of ISSR Molecular Markers. Act. Bot. Hung., *60*(1–2): 47–65.

ESFANDANI-BOZCHALOYI, S., M., SHEIDAI, M., KESHAVARZI, Z., NOORMOHAMMADI (2018b): Species Identification and Population Structure Analysis In *Geranium* Subg. *Geranium* (Geraniaceae) . Hacquetia, *17/2*: 235–246.

ESFANDANI–BOZCHALOYI, S., M., SHEIDAI, M., KESHAVARZI, Z., NOORMOHAMMADI (2018c): Morphometric and ISSR-analysis of local populations of *Geranium molle* L. from the southern coast of the Caspian Sea. Cytology and genetics, *52*, No. 4: 309–321.

ESFANDANI-BOZCHALOYI, S., M., SHEIDAI (2018d): Molecular diversity and genetic relationships among *Geranium pusillum* and *G. pyrenaicum* with inter simple sequence repeat (ISSR) regions, Caryologia, vol. *71*, No. 4: 1-14.

ESFANDANI-BOZCHALOYI, S., M., SHEIDAI (2019): Comparison of DNA Extraction Methods from *Geranium* (Geraniaceae). Acta Botanica Hungarica, *61*(3–4): 251–266.

ESFANDANI-BOZCHALOYI S, SHEIDAI M, KESHAVARZI M, (2018E) MACRO- AND MICRO-MORPHOLOGICAL STUDY OF FRUITS AND SEEDS IN THE GENUS *GERANIUM* (GERANIACEAE), PHYTOTAXA, 375(3):185 – 204. DOI.ORG/10.11646/PHYTOTAXA.375.3.8

ESFANDANI-BOZCHALOYI S, W.ZAMAN (2018f).Taxonomic significance of macro and micro-morphology of *Geranium* L. species Using Scanning Electron Microscopy. Microsc Res Tech,81, 12(652-666). DOI: 10.1002/jemt.23159 october

FREELAND, J.R., H., KIRK, S.D., PETERSON (2011): Molecular Ecology (2nded). Wiley-Blackwell, UK, 449 pp.

HUSON, D.H., D., BRYANT (2006): Application of Phylogenetic Networks in Evolutionary Studies. Molecular Biology and Evolution, *23*: 254−267.

HAMMER, O., D.A., HARPER, P.D., RYAN (2012): PAST: Paleontological Statistics software package for education and data analysis. Palaeonto. Electro., *4:* 9.

HAN, X., Z. WEI, B. ZHANG, Y.LI, T.DU,,... H.CHEN (2021): Crop evapotranspiration prediction by considering dynamic change of crop coefficient and the precipitation effect in back-propagation neural network model. Journal of hydrology (Amsterdam), 596:126104.

HEIKRUJAM, M., J., KUMAR, V., AGRAWAL (2015): Genetic diversity analysis among male and female Jojoba genotypes employing gene targeted molecular markers, start codon targeted (SCoT) polymorphism and CAAT box-derived polymorphism (CBDP) markers. Meta Gene, *5*: 90–97.

JAMZAD, Z. (2012): *Lamiaceae*. – In: Assadi, M., Maassoumi, A. and Mozaffarian, V. (eds): Flora of Iran. Vol. 76. Research Institute of Forests and Rangelands, Tehran, 810 pp.

JIANG, L., B. ZHANG, S. HAN, H. CHEN, & Z.WEI (2021): Upscaling evapotranspiration from the instantaneous to the daily time scale: Assessing six methods including an optimized coefficient based on worldwide eddy covariance flux network. Journal of hydrology (Amsterdam), *596:* 126135.

KNOWLES, L.L. and B., CARSTENS (2007): Delimiting species without monophyletic gene trees. Systematic Biology, *56*: 887-895.

KHARAZIAN, N., S., RAHIMI, B., SHIRAN (2015): Genetic diversity and morphological variability of fifteen *Stachys* (Lamiaceae) species from Iran using morphological and ISSR molecular markers. Biologia, *70*(4):438- 452.

MAYR, E. (1982): The Growth of Biological Thought: Diversity, Evolution, and Inheritance. Cambridge, MA: Harvard University Press, pp.1-992.

MIAO, R., J. MA, Y. LIU, Y. LIU, Z YANG,,... M.GUO (2019): Variability of Aboveground Litter Inputs Alters Soil Carbon and Nitrogen in a Coniferous–Broadleaf Mixed Forest of Central China. Forests, 10(2): 188.

MORENO, S., J.P., MARTIN, J.M., ORTIZ (1998): Inter-simple sequence repeats PCR for characterization of closely related grapevine germplasm. Euphytica, *101*:117–125.

PEAKALL, R., P.E., SMOUSE (2006): GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes, *6*: 288–295.

PELLISSIER, L., J., POTTIER, P., VITTOZ, A., DUBUIS, A., GUISAN (2010): Spatial pattern of floral morphology: possible insight into the effects of pollinators on plant distributions. Oikos, *119*(11): 1805–1813.

PODANI, J. (2000): Introduction to the Exploration of Multivariate Data English translation. Backhuyes publisher, Leide, 407 pp.

PHARMAWATI, M., G.,YAN, I.J., MCFARLANE (2004): Application of RAPD and ISSR markers to analyse molecular relationships in *Grevillea* (Proteaceae). Aust. Syst. Bot., *17*:49–61.

PENG, L., M., RU, B., WANG, Y., WANG, B., LI, J.,YU, Z., LIANG (2014): Genetic diversity assessment of a germplasm collection of *Salvia miltiorrhiza* Bunge. based on morphology, ISSR and SRAP markers. Biochemical Systematics and Ecology, *55*:84-92.

POWELL, W., M., MORGANTE, J.J., DOYLE, J.W., MCNICOL, S.V., TINGEY, A., RAFALSKI (1996): Gene pool variation in genus Glycine subgenus Soja revealed by polymorphic nuclear and chloroplast microsatellites. Genetics, *144*: 793–803.

PATEL, H.K., R,S,, FOUGAT, S., KUMAR, J.G., MISTRY, M., KUMAR (2014): Detection of genetic variation in *Ocimum* species using RAPD and ISSR markers. Biotech, *5*(5):697-707.

PÉREZ-LOSADA, M., J., EIROA, S., MATO, J., DOMÍNGUEZ (2005): Phylogenetic species delimitation of the earth worms *Eiseniafetida* (Savigny, 1826) and *Eiseniaand* Bouché, 1972 (Oligochaeta,Lumbricidae) based on mitochondrial and nuclear DNA sequences. Pedobiologia, *49*: 317–324.

POUR-ABOUGHADAREHA, J., AHMADI, A., MEHRABI, A., ETMINAN, M., MOGHADDAM (2017): Assessment of genetic diversity among Iranian *Triticum* germplasm using agro-morphological traits and start codon targeted (SCoT) markers. Cereal Res. Commun., *45*: 574–586.

POUR-ABOUGHADAREH, A., J., AHMADI, A., MEHRABI, A., ETMINAN, M., MOGHADDAM (2018): Insight into the genetic variability analysis and relationships among some Aegilops and Triticum species, as genome progenitors of bread wheat, using SCoT markers. Plant Biosys. For., *152*: 694–703.

QUE, Y., Y., PAN, Y., LU, C., YANG, Y., YANG, N., HUANG (2014): Genetic analysis of diversity within a Chinese local sugarcane germplasm based on start codon targeted polymorphism. Biomed Res. Int., 1–10.

SONG, Z., X., LI, H.,WANG, J., WANG (2010): Genetic diversity and population structure of *Salvia miltiorrhiza* Bge in China revealed by ISSR and SRAP. Genetica, *138* (2):241-249.

SEPEHRY JAVAN, Z., F., RAHMANI, R., HEIDARI (2012): Assessment of genetic variation of genus *Salvia* by RAPD and ISSR markers. Australian J. Crop Sci., *6*(6):1068-1073.

SIVAPRAKASH, K.R., S.R., PRASANTH, B.P., MOHANTY, A., PARIDA (2004): Genetic diversity of black gram landraces as evaluated by AFLP markers. Curr. Sci., *86*: 1411–1415.

SOUFRAMANIEN, J. and T., GOPALAKRISHNA (2004): A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. TAG, *109*: 1687– 1693.

SHAKOOR, A., Z., FANG, Z., GUL, L., WUYANG, L., XINCAN, S., ESFANDANI-BOZCHALOYI (2021): Morphometric analysis and sequence related amplified polymorphism determine genetic diversity in Salvia species, Notulae Botanicae Horti Agrobotanici Cluj-Napoca, *49*: 12153-12153

TAMS, S.H., A.E., MELCHINGER, E., BAUER (2005): Genetic similarity among European winter triticale elite germplasms assessed with AFLP and comparisons with SSR and pedigree data. Plant Breed., *124*: 154–160.

UDE, G., M., PILLAY, E., OGUNDIWIN (2003): Genetic diversity in an African plantain core collection using AFLP and RAPD markers. TAG, *107*: 248– 255.

WIENS, J.J. (2007): Species Delimitation: New approaches for discovering diversity. Systematic Biology, *56*: 875-878.

WANG, M., J., LI, L., ZHANG, R.W., YANG, C.B., DING, Y.H., ZHOU, Z.Q.,YIN (2011): Genetic diversity among *Salvia miltiorrhiza* Bunge and related species using morphological traits and RAPD markers. J. Med. Plants Res., *5*(13):2687-2694.

WANG, B., Y., ZHANG, C.B.,CHEN, X.L., LI, R.Y., CHEN, L., CHEN (2007): Analysis on genetic diversity of different *Salvia miltiorrhiza* geographical populations in China. Chin. Med. J., *32*:1988–1991.

WANG, O., B., ZHANG, L., LU (2009): Conserved region amplification polymorphism (CoRAP), a novel marker technique for plant genotyping in *Salvia miltiorrhiza*. Plant Mol. Biol. Rep., *27*: 139–143.

WILL, M., R., CLAßEN-BOCKHOFF (2017): Time to split *Salvia* s.l. (Lamiaceae) – new insights from Old World *Salvia* phylogeny. Molecular Phylogenetics and Evolution, *114:* 33–58.

WEISING, K., H., NYBOM, K., WOLFF, G.; KAHL (2005): DNA Fingerprinting in Plants. Principles, Methods, and Applications. 2nd ed. CRC Press, Boca Raton, 472 pp.

WIENS, J.J., T.A., PENKROT (2002): Delimiting species using DNA and morphological variation and discordant species limitsinspinylizards (*Sceloporus*). Systematic Biology, *51*: 69–91.

WALKER, J.B., K.J., SYTSMA, J., TREUTLEIN, M., WINK (2004): Salvia (Lamiaceae) is not monophyletic: implications for the systematics, radiation, and ecological specializations of Salvia and tribe Mentheae. –Amer. J. Bot., *91*(7): 1115–1125.

WANG, L., T. YANG, B. WANG, Q, LIN, S. ZHU, C. LI, F. YU (2020): RALF1-FERONIA complex affects splicing dynamics to modulate stress responses and growth in plants. Science advances, 6(21), z1622.

YOUSEFIAZAR-KHANIAN, M., A., ASGHARI, J., AHMADI (2016): Genetic diversity of *Salvia* species assessed by ISSR and RAPD markers. Not. Bot. Horti. Agrobo., *44*: 431– 436.

ZHANG, W., Y, HU, J, LIU, H. WANG, J. WEI, P.SUN, H.ZHENG (2020): Progress of ethylene action mechanism and its application on plant type formation in crops. Saudi Journal of Biological Sciences, *27*(6): 1667-1673.

ZHANG, B., D. XU, Y. LIU, F. LI, J.CAI, L.DU (2016): Multi-scale evapotranspiration of summer maize and the controlling meteorological factors in north China. Agricultural and Forest Meteorology, *216*: 1-12.

GENETIČKI DIVERZITET I ODNOSI IZMEĐU VRSTA RODA *SALVIA* POMOĆU ISSR MARKERA

Zhao YANPENG[1], Wang HONGMEI[2*], Liang WEI[3], Majid KHAYATNEZHAD[4], FAISAL [5]

[1]Departman za osnovne kurseve, Shandong Univerzitet za nauku i tehnologiju, Taian, Shandong, Kina
[2]Dept. za fiziku, Univerzitet za nauku i tehnologiju, Liaoning, Anshan, Kina
[3]Institut za fiziku, Qingdao Univerzitet za nauku i tehnologiju, Qingdao Shandong 266061, Kina
[4]Departman za spoljašnju sredinu i inžinjerstvo, Islamski Azad Univerzitet, Ardabil, Iran
5 Institute of Plant Sciences, University of Sindh, Jamshoro, Pakistan.

Izvod

Identifikacija vrsta je fundamentalno važna u oblastima zaštite, biologije, biogeografije i ekologije. Vrste žalfije su zeljaste, dvogodišnje ili jednogodišnje, jako aromatične. Molekularni ISSR markeri korišćeni su za procenu genetičkog diverziteta raznolikosti i analize odnosa 30 vrsta žalfije. Deset odabranih ISSR prajmera amplifikovalo je 116 lokusa, od kojih su svi bili polimorfni. Dobijeni prosečni sadržaj informacija o polimorfizmu 0,39, prosečna informativnost traka 10,5 i indeks markera 3,1 otkrivaju visok genetički diverzitet koji preovlađuje među uzorcima *Salvia*. Dendrogram napravljen na osnovu ISSR-a, razdvojio je jedinke u podgrupe u skladu sa njihovim vrstama. Naši rezultati su pokazali da se ISSR markeri mogu koristiti kao pouzdana i informativna tehnika za procenu genetičkog diverziteta i odnosa među vrstama *Salvia*. Ciljevi ove studije su: 1) mogu li ISSR markeri identifikovati vrste *Salvia*, 2) kakva je genetika tim taksonima u Iranu i 3) istražiti međusobni odnos vrsta?

Primljeno 25.III.2020.
Odobreno 18. I. 2021.