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EVALUATION OF GENETIC VARIATION AMONG SATUREJA GENOTYPES USING RAPD MOLECULAR MARKERS AND MORPHOLOGICAL TRAITS

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ABSTRACT

In this study, using morphological traits and 15 RAPD primers, genetic diversity of 24 *satureja* genotypes were assessed. There were significant differences among genotypes in all traits. Based on RAPD data, 100 bands were detected and from these 100 bands, 81 bands were polymorph. The mean numbers of bands were 6.7 per primer. According to similarity matrix of molecular and Euclidean distances of morphological data, similarities ranged between 0.61-0.94 (RAPD marker), and 0.38-092 (morphological traits). Based on molecular data the highest similarities belonged to genotypes number F11, F72 (0.94, RAPD marker). Based on morphological traits, the highest similarities belonged to genotypes number A2, B35 and C11, E6 (92%). Cluster analysis showed that the distinctions based on morphological traits did not confirm the distinction based on molecular data. A dendrogram was prepared on the basis of a similarity matrix using the UPGMA algorithm and separated the 24 genotypes into four groups. Our results also indicate that RAPD approach and morphological analysis seemed to be best-suited for fingerprinting and assessing genetic relationships in Satureja genotypes with high accuracy. results showed that RAPD markers could be useful tools for investigating genetic diversity in species of *Satureja species*p.

Keywords: *Satureja* , Genetic diversity, RAPD molecular marker.

INTRODUCTION

Satureja is a genus of aromatic and medicinal plants of Lamiaceae family (the mint family). The leaves, flowers and stems of Satureja species are used as herbal tea and, in traditional medicine, to treat various ailments, such as cramps, muscle pains, nausea, indigestion, diarrhea and infectious diseases (Gu *et al*., 2003).

Satureja species have become increasingly popular in recent years as plants which can creat "new "oils with unique compositions. There are many similarities between the chemical components of essential oil of Satureja genous and thyme (Sefidkon *et al*., 2010; doosty *et al*., 2012). In Flora Europaea, Heywood & Richardson (Heywood *et al*., 1972) recognized 5 genera in the region including *Calamintha, Acinos, Clinopodium, Micromeria* and *Satureja* . In the Flora of Union of Soviet Socialist Republics (Shishkin *et al*., 1954; China Li *et al.*, 1994). Turkey (Davis, 1982) and Iranica (Rechinger, 1982) a similar classification was adopted considering some specific genera which were included on the basis of geographical distribution. This genus is represented in the flora of Iran by 15 species, nine of which (*S. edmondii*, *S. intermedia*, *S. isophylla*, *S. kallarica*, *S. bachtiarica*, S. *khuzistanica*, *S. atropatana* , *S. macrosiphonia* and *S. sahendica*) being endemic and exclusive to Iran .These are annual or perennial semi-bushy aromatic plants that inhabit arid, sunny, stony and rocky regions (Kahrizi *et al,.* 2007).

They are low-growing herbs and subshrubs, reaching heights of 15–50 cm.The leaves are 1 to 3 cm long, with white to pale pink-violet flowers forming in whorls on the stem. Both summer and winter savories are used to flavor food. The former is preferred by cooks but is only available in summer, the latter however is an evergreen perennial. Certain organic chemicals are derived from these species, which are useful to humans. They are usually well known, and are used by native inhabitants as spice, medicinal plant or source of essential oils. Medicinal properties and a large variety of specimens in the species increase the importance of diverse studies in this genus (Hadian *et al*., 2011). Morphological improvement, biological characters production and modification of the accumulation level of biologically active agents are the most important goals of any medicinal plant breeding program. The variation on the morphological, biological production and pharmaceutical characters has been attributed to environmental and/or genetic factors. DNA technology has recently come to be widely used to estimate the interspecific and intraspecific genome polymorphism and establish the phylogenetic and evolutionary relationships among species. Molecular markers are useful tools in conservation genetic studies (Frankham *et al*., 2000; Mirmoayedi *et al*., 2012). In particular, the polymerase chain reaction (PCR) with arbitrary primers is used to analyze the amplification fragment length polymorphism (AFLP), random amplified polymorphic DNA (RADP), and inter-simple sequence repeats (ISSR). PCR-based

methods with arbitrary primers (Such as RAPD) are mostly used to characterize neutral, unique, and moderately repetitive sequences of the genome (Zietkiewicz *et al*., 1994). In recent years, DNA-based molecular markers have been used to assess the genetic diversity between germplasm in many plant species. DNA-based molecular markers are free from environmental modulations. Random amplified polymorphic DNA (RAPD) markers have proved to be very useful tools, providing a convenient and rapid assessment of the genetic differences between genotypes (Williams *et al.*, 1990). Moreover, RAPDs use arbitrary primers that provide a large number of multilocus markers and can be applied to analyze almost any organism, even those for which no previous genetic or molecular information are available. The RAPD marker, being technically simple and fast (Williams *et al.*, 1990) has been efficiently used to study genetic diversity of different medicinal plant species such as Digitalis obscura L. (Nebauer *et al.,* 1999). Cymbopogon species), (Kapteyn *et al.,* 2002; Cunila galioides Benth Hadian *et al.*, 2010; Sangwan *et al.,* 2011; Rostami-Ahmadvandi *et al.,* 2012).Within the *Satureja* genus the genetic diversity has been dealt with using morphological characters (Dirmenci *et al.,* 2010; Hadian *et al.,* 2011; Kasyani *et al.,* 2012). Enzyme electrophoresis by (Attar *et al*., 2006; Kahrizi *et al.*, 2007; Hadian *et al.*, 2010). chloroplast DNA restriction site analysis (Konstantinos *et al*., 2008). and molecular markers (RAPD,SAMPL and AFLP) (Braüchler *et al*., 2005; Braüchler *et al*., 2006; Braüchler *et al*., 2008; Hadian *et al*., 2008; Hadian *et al*., 2010; Tanyolac b., 2003). The present study was conducted to study, of genetic diversity of different Iranian Satureja genotypes using morphological traits and RAPD markers in order to support the breeding program.

MATERIALS AND METHODS

Plant materials and DNA extraction: Twenty-four genotypes of *Satureja* genus were collected from Research Institute of Forest and Rangelands (Table 1). In each of the twenty-four genotypes, several individuals were selected randomly.For individual plant about five grams of young and clean leaves were sampled, samples were collected and immediately stored in liquid nitrogen for genomic DNA extraction. After collection, the leaves maintained at -80 ◦C.

Table 1. Twenty-four Satureja genotypes materials used in this study.

Code	Genotype name	Code	Genotype name
A2	CHAMGAZ	D37	TAKHTSHAN
A85	CHAMGAZ	E6	ABDANAN
A35	CHAMGAZ	E61	ABDANAN
B35	PAALAM	E70	ABDANAN
B3	PAALAM	F11	LALI
B81	PAALAM	F72	LALI
C11	MAZHIN	G63	CHOVENY
C33	MAZHIN	G55	CHOVENY
C66	MAZHIN	G44	CHOVENY
D26	TAKHTSHAN	H11	MONGERA
D12	TAKHTSHAN	H21	MONGERA
D63	TAKHTSHAN	H24	MONGERA

DNA extraction and PCR amplification: The DNA extraction procedure was based on a CTAB method (Doyle, 1991). The concentration of DNA was determined by UV visible spectrophotometer. DNA samples were stored at -20°C prior to RAPD analysis. DNA quality was evaluated by visual comparison with DNA standards in ethidium bromide-stained agarose/TAE gels (figure 1). PCR amplifications were performed in 25 µl reaction volumes of 10 ng genomic template DNA, 2.5 µl of 10X Taq buffer (Sinagen, Tehran, Iran), 0.5 mM of mixed dNTPs, 0.5 mM primer and 0.25 units of Taq DNA polymeras. Amplification was run on an BioRAD Thermal Cycler (United States) and included initial denaturation at 94°C for 4 min, followed by 5 cycles of denaturation at 92°C for 30 s, primer annealing at Tm-5 °C for 4 min; DNA elongation at 72°C for 5 min, 30-44 °C for 1 min; and last synthesis at 72°C for 6 min. The amplification products were separated in 1.5% agarose gel Ultra pure, Invitrogen) in 1X TAE and were stained with ethidium bromide. 100-1000kb DNA ladder (Fermentas) was used as molecular markers. Out of 18 oligonucleotides primers, 15 were selected according to the number and consistency of amplified fragments (Table 2).

Morphological traits analysis: The seeds of 24 genotypes were planted in propagation pots and then 35 plantlets of each genotype were transplanted to the field with 50 * 30cm spacing in a randomized complete block design (RCBD) with three replications. The investigated

morphological traits were plant height (cm), plant width (cm), branch number main stem length (mm), subsidiary stem length (mm), Spike length(mm), Spikelets length(mm), Peduncle length (mm), Sepals length (mm), Sepals width (mm), Internode length, Stem diameter (mm), Flower length (mm), Leaf length (mm), Leaf width (mm). These traits were evaluated on the basis of 24 individual plants (Ten plants for each replication.

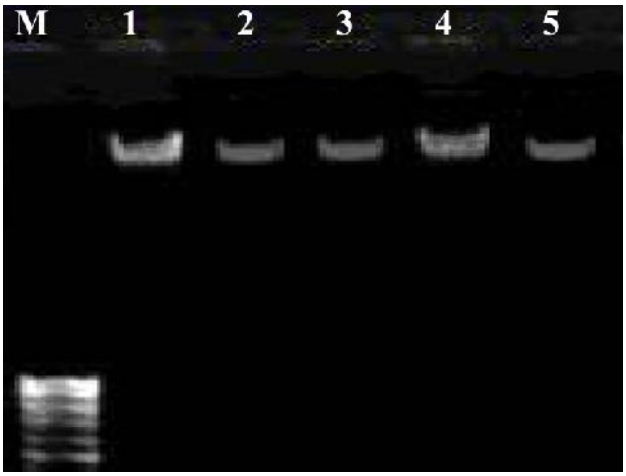

Figure1. Extracted genomic DNA of some *Satureja* genotypes.

Data analysis: Morphological data were analyzed using MSTAT-C. (Mstat-C, 1988) and then means were compared by Duncan multiple range Test at 5% level. Pearson's coefficients were used in order to determine the degree of associations among the characteristics.

In RAPD markers analysis, only reproducible clear and polymorphic were scored, as either present (1) or absent (0) across all accessions (Figure 2). Binary matrix was used to estimate the genetic similarities, by employing Dice index (Nie *et al*., 1979). These similarity coefficients were used to construct dendrogram using the unweighted pair group method with arithmetic averages (UPGMA)

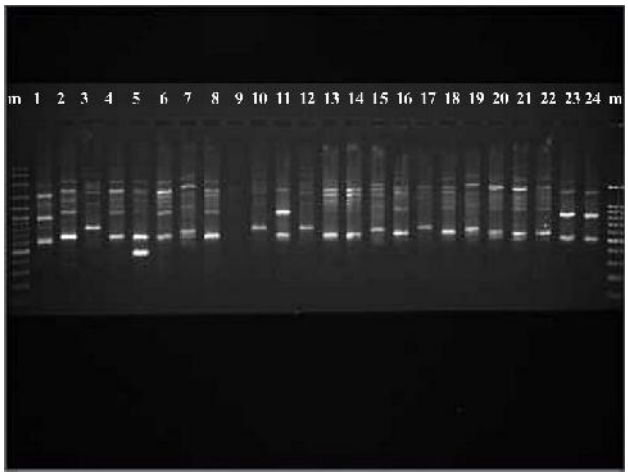

Figure 2. Pattern of 24 *satureja* genotypes by opl16. Lan M: size marker 1Kb

Table 2.Sequence, annealing temperature, total number of bands, number of polymorphic bands and polymorphism percentage of 15 RAPD primers that used in this study.

No	Primer name	Sequence (5–3)	TM (_C)	Total bands	No. of polymorphic band	Percent of polymorphism
1	OPL08	AGCAGGTGGA	36	12	9	75
2	S29	TGATCCCTGG	44	10	9	90
3	OPB07	GGTGACGCAG	38	10	8	80
4	S18	CCACAGCAGT	38	10	10	100
5	OPL16	AGGTTGCAGG	44	7	6	85.71
6	Oligo 213	CAGCG AACTA	30	7	7	100
7	OPB10	CTGCTGGGAC	36	7	5	71.43
8	Oligo 342	GAGATCCCTC	32	6	3	50
9	S32	TCGGCGATAG	34	6	3	50
10	Oligo 349	GGAGCCCCCT	30	5	4	80
11	Oligo 347	TT GCTTGGCG	34	5	5	100
12	OPB11	GTAGACCCGT	40	4	3	75
13	OPF04	GGTGATCAGG	44	4	4	100
14	Oligo 42	TTAACCGGGG	32	4	3	75
15	OPB12	GTAGACCCGT	38	3	2	66.67
Total	-	-	-	100	81	-
Average	-	-	-	6.7	5.4	-

RESULTS AND DISCUSSION

Variability of morphological traits: The results of analysis of variance showed significantly different in all evaluated morphological traits were exist (Table 3).These results suggest that selecting relevant characters can be possible. Means comparison indicated that the highest and lowest plant height were related to A35 (50.33cm) and H24 (23.23 cm)genotypes, respectively that were obtained with density of 20 plants/m^2. Means comparison indicated that the highest and lowest main branch number per plant were C26 (59.6) and H24(2.00) , respectively (Table 4).

The relation between plant highest and main branch number per plant significantly affected plant height but it did not significantly affect the other traits (Table 4). The spike length and spikelete length ranged from 28 to 7 mm and 5.66 to 2.00 (mm), respectively. Genotypes number D63 and E6 had the highest Sepals length (8,00 and 9,00mm, respectively). While, lowest Sepals length (3.66) belonged to genotype, E61. As the results showed, the lowest diversity belonged to trait Sepals width, the highest and lowest, respectively (2.66 - 3.00 and 2.00mm). Means comparison indicated that the highest Internode length belonged to genotype number C66 with value of 11mm and the lowest Internode length belonged to genotypes G44 and F72 (5.66), that it significantly affected other traits (Table 4). The highest and lowest diameter stem (5.00 mm and 2 mm) belonged to genotypes D12 and C33 respectively. Genotypes number A2 and H21 had the highest Leaf length (16.66 mm). While, lowest Leaf length (11.66mm) belonged to genotypes number D11 and E61 with value of 11.66mm. Means comparison indicated that the highest Leaf width (11.33mm) belonged to genotypes F72, and the lowest belonged to G44 with value of (5mm). In the plant height trait, 24 genotypes were divided into 8 groups in which there was no significant difference between theirs while takhtshan (D37) , abdanan (E61), paalam (B3), chamgaz (A85), mazhin (C11), abdanan(E6) and takhtshan(D12) species were in one groups. The highest and lowest height was observed in the chamgaz(A35) and Mongera (H24) genotypes, respectively.

Molecular analysis in Satureja species: The generated band sizes had diverse molecular mass, with the RAPD primers ranging from 350 to 1700 bp. The species exhibited a high level of polymorphism, which was reflected in the number of polymorphic loci (No), and percent polymorphic loci (P). (Table 2).

RAPD analysis: Out of 20 primers 15 were polymorphic, producing 100 high resolution DNA bands, 81 (79.92%) of which were polymorphic among genotypes (Table 2). The number of bands per primer varied from 12 10 (opl08) to 2 (opb12) with an average of 6.7. The average proportion of polymorphic markers across primers was 79.92%, ranging between 50% (*Oligo 342, S32*) and 100% (*OPF04,Oligo 213,Oligo 347,S18*). Estimations of genetic similarity of RAPDs based on the 81 polymorphic markers between 24 cultivated local genotypes of Satureja ranged from 0. 41 for (*B3* and *H24*) to 0.94 for (*F72* and *F11*) (Table 5).

Cluster analysis resulted in classifying the 24 genotypes into four major groups (Fig. 3). A2, A35 and B3 genotypes belongs to first cluster, while genotypes (*A85, E61,E70,F11,F72,C11,C33,C66,E6,G63,G55,G44*,were included in the second cluster and genotypes (*B35,B81,D26,D63,D12,D37*) grouped in the third cluster. The fourth cluster included (*H11,H21, H24*) genotypes. Based on RAPD primers data, F11 and F72 genotypes had the maximum similarity(0.94), while minimum similarity related to B3 and *H24* (0.41).

These results show that RAPD is suitable for genetic diversity assessment in *Satureja species* .

The Obtained results in Genetic diversity based on morphological traits: Cluster analysis resulted in categorizing the 24 genotypes into four major groups. They were divided into four groups based on morphological traits (Figure 4).

The first group included *A2, A35,B3,B35, A85,E61, C33, C11, E6, F11,D26,C66 ,E6,E61, H11,G63, D63,* while the second group included*, F72* and genotypes (*E70, D12, D37*) grouped in the third cluster. the fourth cluster included genotypes (*H21 ,G44, G55*).

Table 3. Analysis of variance of morphological traits.

Leaf width (mm)	Leaf length (mm)	Flower length (mm)	Stem diameter(mm)	Internode length (mm)	Sepals width (mm)	Sepals length (mm)	Peduncle length (mm)	Spikelets length(mm)	Spike length (mm)	subsidiary stem length	branch number per main stem	Plant width (cm)	Plant height (cm)	Degrees of freedom	Sources of change
7.97**	6.43**	6.48**	1.78**	7.48**	0.69**	4.30**	1.82**	3.13**	80.93**	10.24**	876.02**	735.02**	142.48**	23	Treatment
0.68**	0.16**	0.013**	0.097**	0.04**	0.04**	0.18**	0.05**	0.04**	0.43**	0.04**	0.35**	1.17**	1.09**	2	Block
0.46	0.47	0.23	0.39	0.43	0.042	0.09	0.03	0.04	0.57	0.13	0.55	1.64	1.55	46	Error
8.02	4.95	5.97	6.12	8.03	8.03	4.66	6.05	5.57	4.78	10.49	3.31	2.31	.21	71	Coefficient of Variation

Table 4. Results of Duncan's multiple range test for mean comparison of different morphological traits.

Peduncle length	Spikelets length	Spike length	subsidiary stem length	branch number per main stem	Plant width	Plant height	Genotype name
4.00 B	3.00 D	1 7.33 EF	2.00 E	30.33 E	50.66 KJ	39.33 E	Chamgaz(A2)
2.00 E	4.66 B	8.66 J	3.00 D	44.66 D	63.66 F	45.33 B	Chamgaz(A85)
4.00 B	4.00 C	17.33 EF	7.66 A	10.33 K	71.33 D	50.33A	Chamgaz(A35)
3.00 C	3.00 D	23.00 B	3.00 D	19.66 H	58.00 H	40.00 DE	Paalam(B35)
4.33 A	4.00 C	12.33 H	3.00 D	26.33 F	73.33 DC	45.66 B	Paalam(B3)
2.00 E	4.00 C	16.00 FG	3.00 D	29.66 E	48.66 K	42.33 C	Paalam(B81)
3.00 C	4.00 C	17.33 EF	2.00 E	59.66 A	77.66 B	45.00 B	Mazhin(C26)
2.00 E	3.00 D	13.00H	2.00 E	29.33 E	58.66 H	40.00 DE	Mazhin(C33)
3.00 C	4.00 C	20.33 C	3.00 D	30.66 E	73.66 C	42.00 DC	Mazhin(C66)
3.00 C	5.00 B	22.66 B	6.66 B	7.66 L	41.00 M	39.66 E	Takhtshan(D26)
2.00 E	2.00 E	15.66 G	4.00 C	15.66 J	66.33 E	44.66 B	Takhtshan(D12)
3.00 C	4.00 C	7.00 K	2.00 E	24.33 G	51.33 J	30.66 GF	Takhtshan(D63)
2.00 E	3.00 D	15.33 G	1.00 F	55.33 B	61.33 G	46.33 B	Takhtshan(D37)
4.00 B	5.00 B	19.66 CD	2.00 E	49.33 C	95.33 A	44.66 B	Abdanan(E6)
2.00 E	4.00 C	20.33 C	2.00 E	20.66 H	54.00 I	46.33 B	Abdanan(E61)
3.00 C	5.66 A	28.00 A	2.00 E	29.33 E	62.00 GF	38.66 E	Abdanan(E70)
2.00 E	2.00 E	7.00 K	2.00 E	17.66 I	50.66 KJ	32.33 GF	Lali(F11)
2.00 E	2.00 E	11.66 HI	4.00 C	9.33 K	41.00 M	33.00 F	Lali(F72)
3.00 C	4.00 C	18.00 E	7.00 B	3.00 NO	38.33 N	33.00 F	Choveny(G63)
2.00 E	2.00 E	18.66 DE	3.00 B	5.66 M	43.66 L	32.66 F	Choveny(G55)
2.00 E	3.00 D	11.66 HI	4.00 C	5.00 M	44.33 L	31.00 GF	Choveny(G44)
3.00 C	4.00 C	12.66 H	3.66 CD	5.33 M	32.66 O	32.66 F	Mongera(H11)
2.00 E	4.66 B	16.33 FG	3.00 D	3.66 N	33.00 O	30.33 G	Mongera(H21)
2.33 D	4.00 C	10.66 I	7.00 B	2.00 O	39.33 MN	23.33 H	Mongera(H24)
Leaf width	**Leaf length**	**Flower length**	**Stem diameter**	**Internode length**	**Sepals width**	**Sepals length**	**Genotype name**
10.66 AB	16.66 A	5.66 I	4.00 C	7.66EFGH	2.00B	7.00 DE	Chamgaz(A2)
8.66 DEF	14.33 CD	8.66 DH	4.66 B	10.66 AB	3.00 A	6.00 F	Chamgaz(A85)
8.66 DEF	14.66 CD	8.00EF	3.00 DE	9.66 BC	2.00 B	6.00 F	Chamgaz(A35)
8.00 EFG	16.33 AB	11.66 A	4.00 C	7.66 EFGH	2.00 B	6.66 E	Paalam(B35)
6.66 H	12.33 FG	7.66 FG	4.00 C	7.33 FGHI	2.00 B	7.00 DE	Paalam(B3)
8.33 EF	14.33 CD	7.00 GH	3.00 DE	9.00 CD	2.66 A	6.00 F	Paalam(B81)
7.66 FGH	12.33 FG	10.00 B	3.00 DE	10.33 AB	3.00 A	6.00 F	Mazhin(C26)
10.66 AB	15.33 BC	8.33 DEF	2.00 F	11.00 A	3.00 A	7.00 DE	Mazhin(C33)
8.66 DEF	14.00 D	9.00 CD	4.00 C	7.66 EFGH	3.00 A	5.00 G	Mazhin(C66)
7.66 FGH	12.66 EFG	8.00 EF	3.00 DE	7.00 GHI	3.00 A	7.00 DE	Takhtshan(D26)
10.00 BC	11.66 G	7.66 FG	5.00 A	9.66 BC	3.00 A	5.00 G	Takhtshan(D12)
8.66 DEF	15.33 BC	4.66 J	3.00 DE	8.33 DEF	2.00 B	9.00 A	Takhtshan(D63)
10.00 BC	12.00 G	7.66 FG	3.00 DE	8.66 CDE	3.00 A	6.00 F	Takhtshan(D37)
10.66 AB	13.66 DE	8.66 DE	4.00 C	10.33 AB	3.00 A	8.66 A	Abdanan(E6)
8.66 DEF	11.66 G	9.00 CD	3.00 DE	8.33 DEF	2.00 B	3.66 H	Abdanan(E61)
7.66 FGH	12.66 EFG	8.66 DE	3.00 DE	6.66 GHI	2.66 A	6.66 E	Abdanan(E70)
8.33 EF	13.66 DE	8.66 DE	2.00 F	6.33 IJ	2.00 B	5.00 G	Lali(F11)
11.33 A	13.33 DEF	5.66 I	2.00 F	5.66 J	3.00 A	7.00 DE	Lali(F72)
9.00 CDE	33.66 DE	8.66 DE	3.00 DE	9.66 BC	3.00 A	6.66 E	Choveny(G63)
10.66 AB	14.00 D	9.66 BC	3.00 DE	7.00 GHI	2.00 B	6.00 F	Choveny(G55)
11.33 A	13.33 DEF	8.00 EF	2.66 E	5.66 J	2.00 B	7.33 CD	Choveny(G44)
7.00 GH	14.66 CD	7.66 FG	3.00 DE	8.00 DEFG	3.00 A	8.00 B	Mongera(H11)
9.66 BCD	16.66 A	8.00 EF	3.00 DE	7.00 HI	2.00 B	7.00 DE	Mongera(H21)
7.00 GH	13.66 DE	6.66 H	3.33 D	6.6 HIJ	2.66 A	7.66 BC	Mongera(H24)

Leaf width	Leaf length	Flower length	Stem diameter	Internode length	Sepals width	Sepals length	Genotype name
10.66 AB	16.66 A	5.66 I	4.00 C	7.66EFGH	2.00B	7.00 DE	Chamgaz(A2)
8.66 DEF	14.33 CD	8.66 DH	4.66 B	10.66 AB	3.00 A	6.00 F	Chamgaz(A85)
8.66 DEF	14.66 CD	8.00EF	3.00 DE	9.66 BC	2.00 B	6.00 F	Chamgaz(A35)
8.00 EFG	16.33 AB	11.66 A	4.00 C	7.66 EFGH	2.00 B	6.66 E	Paalam(B35)
6.66 H	12.33 FG	7.66 FG	4.00 C	7.33 FGHI	2.00 B	7.00 DE	Paalam(B3)
8.33 EF	14.33 CD	7.00 GH	3.00 DE	9.00 CD	2.66 A	6.00 F	Paalam(B81)
7.66 FGH	12.33 FG	10.00 B	3.00 DE	10.33 AB	3.00 A	6.00 F	Mazhin(C26)
10.66 AB	15.33 BC	8.33 DEF	2.00 F	11.00 A	3.00 A	7.00 DE	Mazhin(C33)
8.66 DEF	14.00 D	9.00 CD	4.00 C	7.66 EFGH	3.00 A	5.00 G	Mazhin(C66)
7.66 FGH	12.66 EFG	8.00 EF	3.00 DE	7.00 GHI	3.00 A	7.00 DE	Takhtshan(D26)
10.00 BC	11.66 G	7.66 FG	5.00 A	9.66 BC	3.00 A	5.00 G	Takhtshan(D12)
8.66 DEF	15.33 BC	4.66 J	3.00 DE	8.33 DEF	2.00 B	9.00 A	Takhtshan(D63)
10.00 BC	12.00 G	7.66 FG	3.00 DE	8.66 CDE	3.00 A	6.00 F	Takhtshan(D37)
10.66 AB	13.66 DE	8.66 DE	4.00 C	10.33 AB	3.00 A	8.66 A	Abdanan(E6)
8.66 DEF	11.66 G	9.00 CD	3.00 DE	8.33 DEF	2.00 B	3.66 H	Abdanan(E61)
7.66 FGH	12.66 EFG	8.66 DE	3.00 DE	6.66 GHI	2.66 A	6.66 E	Abdanan(E70)
8.33 EF	13.66 DE	8.66 DE	2.00 F	6.33 IJ	2.00 B	5.00 G	Lali(F11)
11.33 A	13.33 DEF	5.66 I	2.00 F	5.66 J	3.00 A	7.00 DE	Lali(F72)
9.00 CDE	33.66 DE	8.66 DE	3.00 DE	9.66 BC	3.00 A	6.66 E	Choveny(G63)
10.66 AB	14.00 D	9.66 BC	3.00 DE	7.00 GHI	2.00 B	6.00 F	Choveny(G55)
11.33 A	13.33 DEF	8.00 EF	2.66 E	5.66 J	2.00 B	7.33 CD	Choveny(G44)
7.00 GH	14.66 CD	7.66 FG	3.00 DE	8.00 DEFG	3.00 A	8.00 B	Mongera(H11)
9.66 BCD	16.66 A	8.00 EF	3.00 DE	7.00 HI	2.00 B	7.00 DE	Mongera(H21)
7.00 GH	13.66 DE	6.66 H	3.33 D	6.6 HIJ	2.66 A	7.66 BC	Mongera(H24)

Leaf width	Leaf length	Flower length	Stem diameter	Internode length	Sepals width	Sepals length	Genotype name
10.66 AB	16.66 A	5.66 I	4.00 C	7.66EFGH	2.00B	7.00 DE	Chamgaz(A2)
8.66 DEF	14.33 CD	8.66 DH	4.66 B	10.66 AB	3.00 A	6.00 F	Chamgaz(A85)
8.66 DEF	14.66 CD	8.00EF	3.00 DE	9.66 BC	2.00 B	6.00 F	Chamgaz(A35)
8.00 EFG	16.33 AB	11.66 A	4.00 C	7.66 EFGH	2.00 B	6.66 E	Paalam(B35)
6.66 H	12.33 FG	7.66 FG	4.00 C	7.33 FGHI	2.00 B	7.00 DE	Paalam(B3)
8.33 EF	14.33 CD	7.00 GH	3.00 DE	9.00 CD	2.66 A	6.00 F	Paalam(B81)
7.66 FGH	12.33 FG	10.00 B	3.00 DE	10.33 AB	3.00 A	6.00 F	Mazhin(C26)
10.66 AB	15.33 BC	8.33 DEF	2.00 F	11.00 A	3.00 A	7.00 DE	Mazhin(C33)
8.66 DEF	14.00 D	9.00 CD	4.00 C	7.66 EFGH	3.00 A	5.00 G	Mazhin(C66)
7.66 FGH	12.66 EFG	8.00 EF	3.00 DE	7.00 GHI	3.00 A	7.00 DE	Takhtshan(D26)
10.00 BC	11.66 G	7.66 FG	5.00 A	9.66 BC	3.00 A	5.00 G	Takhtshan(D12)
8.66 DEF	15.33 BC	4.66 J	3.00 DE	8.33 DEF	2.00 B	9.00 A	Takhtshan(D63)
10.00 BC	12.00 G	7.66 FG	3.00 DE	8.66 CDE	3.00 A	6.00 F	Takhtshan(D37)
10.66 AB	13.66 DE	8.66 DE	4.00 C	10.33 AB	3.00 A	8.66 A	Abdanan(E6)
8.66 DEF	11.66 G	9.00 CD	3.00 DE	8.33 DEF	2.00 B	3.66 H	Abdanan(E61)
7.66 FGH	12.66 EFG	8.66 DE	3.00 DE	6.66 GHI	2.66 A	6.66 E	Abdanan(E70)
8.33 EF	13.66 DE	8.66 DE	2.00 F	6.33 IJ	2.00 B	5.00 G	Lali(F11)
11.33 A	13.33 DEF	5.66 I	2.00 F	5.66 J	3.00 A	7.00 DE	Lali(F72)
9.00 CDE	33.66 DE	8.66 DE	3.00 DE	9.66 BC	3.00 A	6.66 E	Choveny(G63)
10.66 AB	14.00 D	9.66 BC	3.00 DE	7.00 GHI	2.00 B	6.00 F	Choveny(G55)
11.33 A	13.33 DEF	8.00 EF	2.66 E	5.66 J	2.00 B	7.33 CD	Choveny(G44)
7.00 GH	14.66 CD	7.66 FG	3.00 DE	8.00 DEFG	3.00 A	8.00 B	Mongera(H11)
9.66 BCD	16.66 A	8.00 EF	3.00 DE	7.00 HI	2.00 B	7.00 DE	Mongera(H21)
7.00 GH	13.66 DE	6.66 H	3.33 D	6.6 HIJ	2.66 A	7.66 BC	Mongera(H24)

Means followed by the same letters in each column-according to Duncan's multiple range test are not significantly ($P<0.05$)

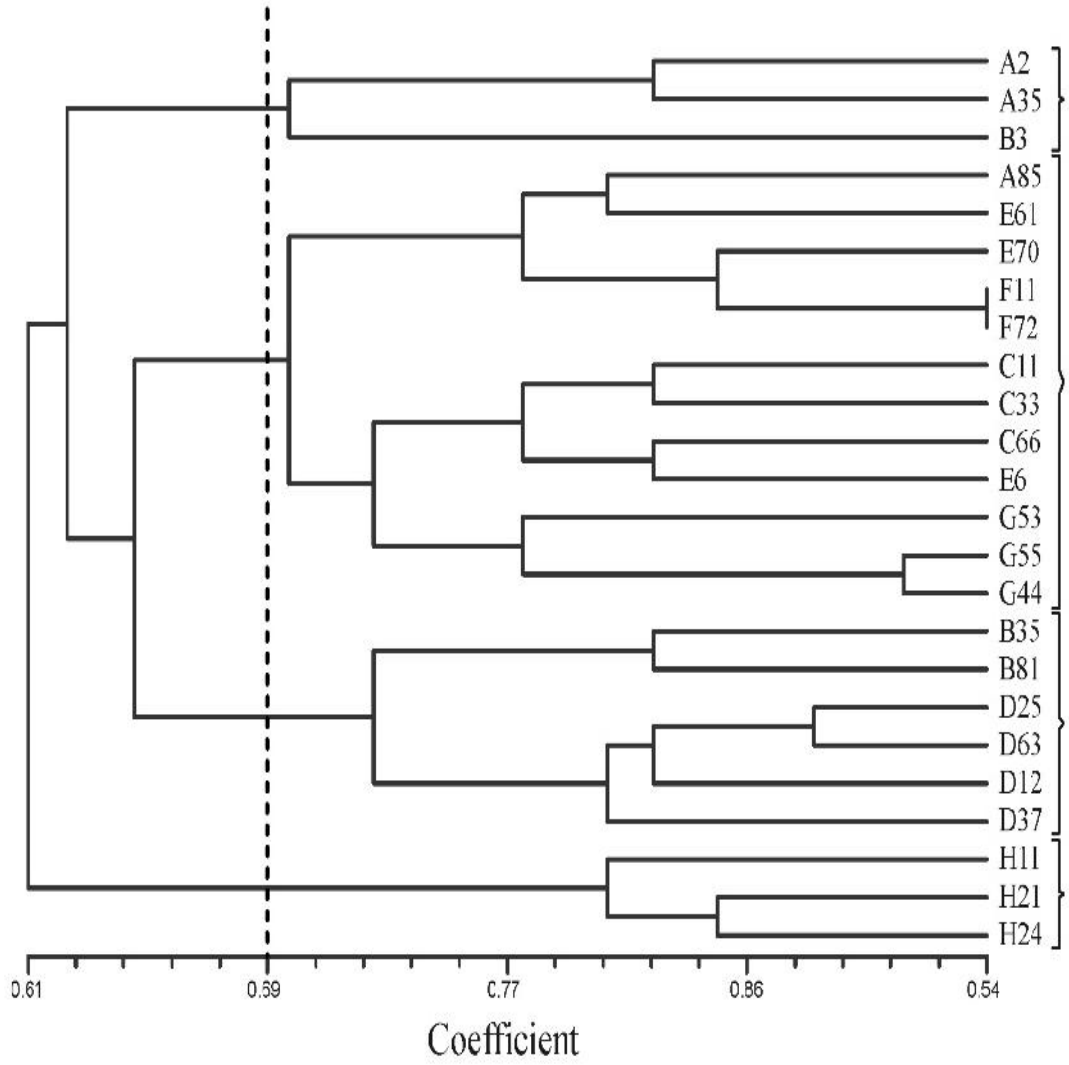

Figure 3. UPGMA dendrogram showing genetic relationships among the 24 genotypes of Satureja species

Table 5. Similarity matrix among Saturja. sp genotypess by Nei and Li's coefficient based on RAPD bands

G	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	**21**	**22**	**23**	**24**
1	**1**																							
2	0.67	**1**																						
3	0.82	0.72	**1**																					
4	0.64	0.68	0.63	**1**																				
5	0.67	0.64	0.72	0.63	**1**																			
6	0.65	0.67	0.71	0.82	0.80	**1**																		
7	0.62	0.73	0.62	0.58	0.59	0.69	**1**																	
8	0.71	0.70	0.72	0.68	0.71	0.76	0.83	**1**																
9	0.70	0.62	0.72	0.66	0.61	0.66	0.72	0.78	**1**															
10	0.60	0.57	0.71	0.70	0.70	0.80	0.65	0.67	0.75	**1**														
11	0.66	0.61	0.66	0.74	0.69	0.80	0.83	0.79	0.77	0.84	**1**													
12	0.58	0.57	0.61	0.71	0.53	0.70	0.59	0.61	0.76	0.88	0.82	**1**												
13	0.45	0.63	0.41	0.65	0.68	0.74	0.61	0.58	0.62	0.80	0.79	0.83	**1**											
14	0.71	0.69	0.66	0.72	0.57	0.71	0.67	0.83	0.82	0.66	0.76	0.72	0.67	**1**										
15	0.61	0.80	0.74	0.53	0.53	0.58	0.66	0.68	0.74	0.73	0.59	0.69	0.67	0.68	**1**									
16	0.58	0.73	0.58	0.67	0.65	0.71	0.66	0.72	0.56	0.60	0.61	0.56	0.74	0.74	0.71	**1**								
17	0.63	0.81	0.72	0.66	0.54	0.65	0.63	0.81	0.77	0.69	0.63	0.60	0.60	0.75	0.88	0.82	**1**							
18	0.47	0.67	0.55	0.70	0.54	0.67	0.63	0.79	0.62	0.59	0.61	0.56	0.67	0.64	0.77	0.88	0.94	**1**						
19	0.53	0.63	0.58	0.66	0.55	0.68	0.73	0.75	0.52	0.58	0.65	0.59	0.58	0.61	0.69	0.71	0.67	0.83	**1**					
20	0.60	0.73	0.68	0.79	0.54	0.71	0.70	0.82	0.76	0.62	0.66	0.60	0.53	0.79	0.64	0.73	0.76	0.84	0.90	**1**				
21	0.70	0.71	0.67	0.67	0.60	0.68	0.67	0.78	0.73	0.59	0.63	0.54	0.43	0.80	0.67	0.66	0.74	61	0.65	0.91	**1**			
22	0.60	0.79	0.64	0.64	0.65	0.65	0.60	0.67	0.58	0.48	0.57	0.50	0.61	0.54	0.65	0.65	0.70	0.70	0.58	0.62	0.66	**1**		
23	0.57	0.61	0.64	0.70	0.50	0.59	0.49	0.63	0.73	0.61	0.53	0.61	0.57	0.66	0.70	0.59	0.77	0.70	0.54	0.68	0.66	0.78	**1**	
24	0.56	0.60	0.48	0.61	0.41	0.59	0.49	0.62	0.62	0.52	0.46	0.59	0.53	0.51	0.68	0.66	0.86	0.82	0.64	0.66	0.54	0.81	0.85	**1**

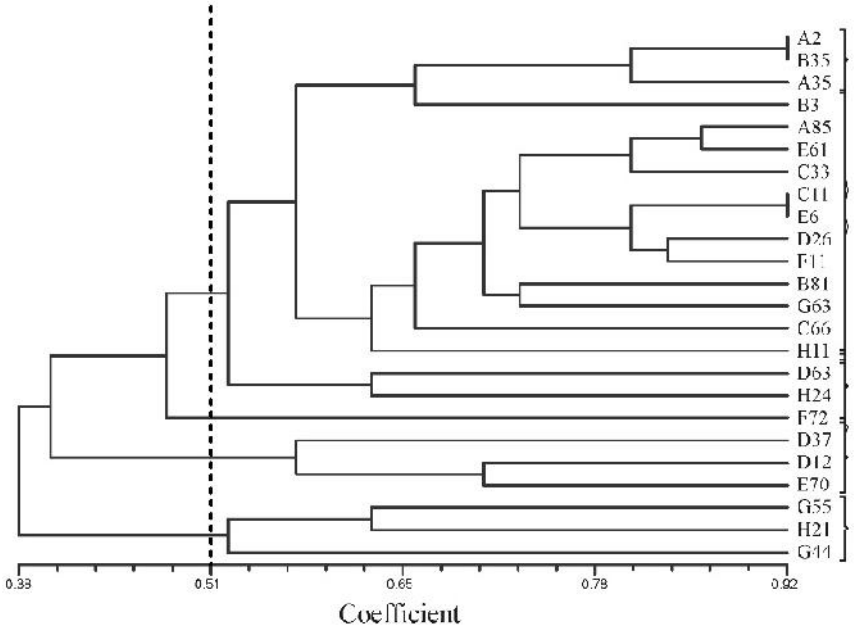

Figure 4. Dendrogram of satureja genotypes using ward method based on Euclidian distance for morphological traits.

DISCUSSION

Genetic variation is a basic requirement for plant breeding, whereas a high genetic variation is needed for genetic improvement of plants. Exploration and evaluation of diversity among and within populations would be of great significance for in situ conservation and *Satureja species* breeding programs. In recent years, genetic markers have been increasingly used to study genetic diversity. Moreover, the polymorphism determined by these markers is one of the valuable parameters for studying populations and understanding their genetic differences. The Savory landraces create a rich source of biodiversity and to better conserve and utilize them their genetic structure should be well characterized and understood. DNA fingerprinting is a routine method employed to study the extent of genetic diversity across a set of germplasm or cultivars and to group them into specific categories.

As mentioned in the results, fourteen morphological traits in twenty four genotypes of satureja species include plant height, Plant width, branch number per main stem, subsidiary stem length, Spike length, Spikelets length, Peduncle length, Sepals length, Sepals width, Internode length, Stem diameter, Flower length, Leaf length and Leaf width are discussed. Some morphological traits for instance Plant width and branch number per main stem have highest degree of genetic variation and classified into 15 groups in which significantly difference was observed while some of these morphological traits for instance Sepals width, Internode length, Leaf length and Leaf width have lowest degree of genetic variation and classified into 2 and 3 groups in which significantly difference was observed. Some morphological traits for instance Spikelets length, Peduncle length, plant height, subsidiary stem length, Sepals length, Stem diameter and Flower length have moderate degree of genetic variation and classified into 5 and 6 groups in which significantly difference was observed (Table 4). Literature review indicated that the genetic diversity based on morphological traits in *Satureja species* has not been done yet.

There is much environmental influence accounting for the morphological traits variability observed. Therefore, when compared to RAPD techniques, Morphological traits are relatively less reliable and inefficient for precise discrimination of closely related genotypes and analysis of their genetic similarities. Successful management and preservation of populations of threatened species depend on a good understanding of the distribution of genetic variation in the species (Francisco-Ortega *et al*., 2000; Wallace, 2002).

Comparative studies in *Satureja species* involving RAPD, AFLP, and SAMPL marker systems have been used by very limited researchers (Hadian *et al*., 2008; Hadian *et al*., 2010). The discriminative power of DNA markers used as a tool to characterize the *Satureja* genus is very important because they can be used to assess the genetic diversity among the *Satureja species* and populations.

Clustering analysis using Ward algorithm based on Nei's Unbiased Measures of Genetic distance, classified the *Satureja* genotypes into four major groups. The cophenetic correlation of Ward was about (r = 0.95). The rate of genetic affinities and relatedness can also be observed between the 24 genotypes (Fig. 2). RAPD primers generated 3 to 12 bands with average of 6.7 bands per populations. The distribution of different microsatellite sequences in different populations determines the possibility of using this method for DNA fingerprinting. (Hadian *et al*., 2008). detected 83 % of polymorphism in 28 accessions of *Satureja* hortensis L., collected from different parts of Iran with RAPDs. (Hadian *et al*., 2008). Phylogenetic relationships and genetic diversity of different Iranian *Satureja species* using AFLP and SAMPL markers also reported (Hadian *et al*., 2008; Hadian *et al*., 2010). Classification of diversity in germplasm collections is important for both plant breeding and germplasm collection. The choice of genetic diversity estimation will largely depend on tools available to the researcher and how they fit into the breeding scheme. In this study, we investigated genetic diversity in 24 Iranian genotypes of *Satureja* based on horticultural characteristics and RAPD markers.

Some differences and similarities were observed by examining the dendrogram obtained from morphological characteristic RAPD markers. For example, genotypes (types *F11* and *F72*) of *Satureja* were most similar to the grouping of molecular data while the two genotypes in the dendrogram obtained from morphological characters were far from each other. Given dendrogram correlation Coefficient (r=0.95). There was significant and negative correlation between similarity matrices of molecular data and morphological traits. However, morphological traits are useful for preliminary, fast, simple, and inexpensive varietals identifications and can be used as a general approach to assess genetic diversity among accessions. In addition, since most of the morphological traits are controlled by many genes, they are strongly influenced by environmental conditions and sequences of RAPD molecular markers are randomly distributed throughout the genome (even off points of the genome). They mainly have no phenotypic occurrence that would seem reasonable (Dey *et al*., 2006). Our findings would provide important genetic information for developing conservation strategies and cultivation of Satureja species. RAPD-PCR gave complete, reliable, reproducible and highly polymorphic fingerprints within and among populations of *Satureja species*.

Moreover, although the RAPD marker is widely used in the studies of samples classification and detection of cultivars and genetic diversity (Konstantinos *et al*., 2008). its repeatable feature has always been questioned. One of the reasons for this issue is the use of short primers and low temperature to connect primer to the DNA tamplet. This causes non-specific and random amplification in some areas that are only slightly similar. Two solutions are proposed for this problem:a) Replication of the experiment in the same situation and removal of unrepeatable bands. b) Conducting only one test and accepting a percentage of error (Anju *et al*., 2006). However, since the main part of genome consisted of non-coding parts, another reason for the lack of correlation between the two dendrograms was distribution of selected RAPD primers at all levels of genomes Mc (Clean *et al*., 2009). This method with high accuracy is suitable for fingerprinting and assessment of genetic relationships in *Satureja* species (Hadian *et al*., 2008). According to results obtained in this study, molecular RAPD marker is a useful tool in the evaluation of the molecular genetic relationships of *Satureja species*.

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