

Diversity in Cucumber Genotypes Based on Morphological Traits and SSR Molecular Markers

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Biodiversity is one of the most important factors in the survival and improvement of any species. Therefore, germplasm collection is the first step for plant improvement. To investigate their genetic and morphological relationships, 10 morphological traits of 20 genotypes of local cucumbers were evaluated using 9 SSR primers. A high genetic variability was observed for the number of flowers per plant. The values of the Jaccard similarity coefficient ranged between 0.51 and 0.92, indicating a high diversity of the genotypes. To evaluate the genetic similarity among genotypes, a cluster analysis using the UPGMA method was performed based on the Jaccard similarity coefficient. The average genetic distance between genotypes (using the Jaccard similarity coefficient) was 0.74 and the mean polymorphic information content (PIC) was 0.69. The primer SSR13251 had the highest PIC (0.8). The clustering pattern of the SSR markers did not coincide with the groupings based on quantitative traits. A dendrogram of the cluster analysis of molecular data showed a high diversity among the studied genotypes. The highest genetic similarity was between genotypes 2 and 3 (0.94), and the lowest genetic similarity was between genotypes 6 and 12 (0.51). The results suggest that SSR markers are a suitable tool to study the genetic diversity and relationships among different genotypes in cucumber.

Keywords: Genetic relationship, Dendrogram, Cucumber, SSR Molecular marker.

Cucumbers ($2n=2x=14$), belonging to the Cucurbitaceae family, are one of the most important vegetables (Jeffery, 1980). They can be used in salads or in processed forms (pickled, salty). The primary origins of diversity cucumber varieties have reported India (Wehner and Robinson, 1991). The global production of cucumbers was 57.55 million tons in 2010. The study of genetic diversity examines the difference or similarity between species, populations or individuals

using special statistical methods and models based on morphological characteristics, stemma information or molecular features of individuals (Bommineni *et al.*, 1997). The higher the genetic diversity is in a community, the wider the choice domain will become and this genetic diversity is the basis for all selections (Pitrat M., 1991). There are different methods used to study the genetic diversity and structure of plant genotypes. Studying morphological traits is a classic example; however, given that the number of morphological traits is limited and is influenced by the age of the plant and the environment, more specific methods, such as molecular markers, are used at present (Fares *et al.*, 2009). Previous studies have shown the genetic

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diversity of cucumber varieties using physiological parameters, fruit characteristics and molecular markers. For instance, Simple sequence repeat (SSR) and Inter simple sequence repeat (ISSR) markers have been used to determine the genetic diversity and fingerprints of cucumber and melon varieties. (Pandey *et al.*, 2013; Parvathaneni *et al.*, 2011). Sequence-related amplified polymorphism (SRAP) and Random amplified polymorphic DNA (RAPD) are additional molecular markers that have been used to determine the genetic diversity of cantaloupe varieties (Yildiz *et al.*, 2011). Microsatellite markers, the second generation of DNA markers, are commonly used due to their high diversity, frequency, repeatability, Mendelian inheritance and codominant nature. Microsatellites are repeated simple sequences or a highly varied sequence of arrays of short motif (1-6 nucleotides) markers. Microsatellite markers can be used to analyze the genetic structure of populations, stemma analysis and kinship determination, and location of controller genes of quantitative traits (Li *et al.*, 2000). Most known microsatellites in one species can be used in other species and this shows their transmissibility (Wunsch 2009). SSR markers have a large potential for identifying polymorphism and heterozygosity in one species, making it possible to distinguish many alleles at one gene locus (Pawell, 1996). Genetic diversity in cucumber has been studied with molecular markers including RAPD (Horejsi and Staub, 1999), AFLP (Li *et al.*, 2004), ISSR ((Wang *et al.*, 2007, SSR (Danin-Poleg *et al.*, 2001),) and EST-SSR (Hu *et al.*, 2010).

In general, local varieties of Iranian cucumbers are not often cultivated due to their low performance and quality. Large quantities of expensive commercial seeds are imported annually into Iran. Additionally, the neglect of local varieties is eliminating them, subsequently eliminating genetic resources that are often compatible with Iran's climates. For this reason, the identification and restoration of local Iranian cucumber varieties is essential for use in the country's future eugenic programs. The aim of this study is to investigate the morphological and molecular diversity of a number of local cucumber genotypes, to determine the most appropriate genotypes for heterosis

and hybridization and to evaluate the marker performance in separating the genotypes.

MATERIALS AND METHODS

Plant materials and extraction of DNA

In this study, 20 genotypes of cucumber were used (Table 1). DNA extraction of all genotypes was done from young, non-developed leaves using the cetyl trimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987).

Data Collection

The experimental design was randomized completed block (RCBD) with three replications. The distance between rows was 50 cm and the distance between plants was 40 cm. Each replicate included ten plants. Five plants were randomly selected and data on ten quantitative traits were recorded (including root length, plumule length, number of primary branches per plant, number of days from three-leaf to six-leaf stage, the number of flowers per plant, number of days until the beginning of flowering and plant height).

PCR Reaction and SSR Analysis

The quantity and quality of extracted DNA was examined through spectrophotometry at wavelengths 260 and 280 nm, and agarose gel electrophoresis was determined at one percent. A polymerase chain reaction was conducted using nine pairs of primers (Table 2). The thermocycler program was the following: one denaturation cycle for 94 min at 4°C, 36 cycles including denaturation at 94°C for one minute and connection of primers to pattern strings, depending on the type of primer, at 50-58°C for one minute, extension at 72 °C for one minute and a final extension cycle was performed for ten minutes at 72°C. The separation of reaction products was accomplished using agarose gel (2.5%) and staining was done with ethidium bromide. Polymorphic patterns, based on the presence or absence of bands, were scored one and zero, respectively. The cluster analysis of the data, based on the Jaccard similarity coefficient, using the Unweighted pair group method with arithmetic mean (UPGMA) method was conducted using the software NTSYSpc (ver. 2.02). The polymorphic information content of each SSR marker was calculated with the following formula: $PIC = 1 - \sum p_i^2$ (Manifesto *et al.*, 2001).

RESULTS

Morphological analysis

Table 3 presents details of the ten morphologic traits observed. The magnitude of changes to the domain emphasized the considerable

Table 1. Studied genotypes and their origins

No.	Genotype	Origin
Abadeh (Fars)	TN-141-94	1
Sarband (Markazi)	TN-154-94	2
Orumieh (West Azarbajejan)	TN-163-94	3
Nahavand (Hamedan)	TN-166-94	4
Sarband (Markazi)	TN-180-94	5
Minab (Hormozgan)	TN-186-94	6
Markazi	TN-200-94	7
Taft (Yazd)	TN-204-94	8
Mazandaran	TN-206-94	9
Baneh (Kordestan)	TN-211-94	10
Sanandaj (Kordestan)	TN-215-94	11
Orumieh (West Azarbajejan)	TN-218-94	12
Kankan (Booshehr)	TN-221-94	13
Esfahan	TN-226-94	14
Noshahr (Mazandaran)	TN-229-94	15
Varamin (Tehran)	TN-244-94	16
Roodbar (Gilan)	TN-250-94	17
Roodbar (Gilan)	TN-251-94	18
Zanjan (Zanjan)	TN-262-94	19
Neishaboor (KhorasanRazavi)	TN-264-94	20

diversity in most of the observed traits. Number of flowers per plant had a change to the domain of 30, which was the largest change in the studied genotypes. Plant height, with an average of 22.56, and number of primary branches, with an average of 2.33, were the second and third largest changes. The smallest changes were for the number of days to the beginning of flowering, number of days to the 4-leaf stage and number of days to the 6-leaf stage. The number of flowers per plant in genotype one was maximum and equal to 50. the root length in genotype six had the highest value with 7.9 cm. The plant height in genotype 1 was maximum and equal to 35 cm. The variance analysis showed that the difference among genotypes for all traits was significant for genetic diversity among the studied genotypes. Most of traits had significant genetic diversity at a probability level of 99%; only the number of primary branches per plant had significant genetic diversity at a probability level of 95%. The variation coefficient was less than 30 in most of the studied traits, which indicates experimental accuracy (Table 4).

Cluster analysis based on quantitative traits

Using the application 2.02 NTSYS, a dendrogram was drawn and the genotypes were clustered into three main groups (Figure 1). The first cluster included genotypes 1, 7 and 20, the second cluster included seven genotypes, and the third cluster included eleven genotypes.

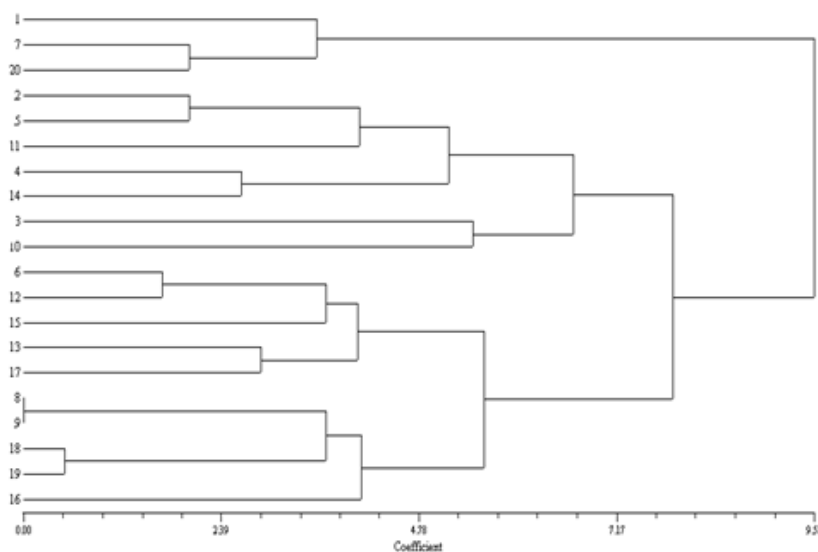


Fig. 1. The dendrogram of the analysis of morphological traits studied in local cucumber genotypes (*Cucumis sativus*)

Table 2. Properties of microsatellite markers used in this study

Primer	Sequence	number of alleles	percentage of Polymorphism	Length	optimum connection temperature	Motif
SSR12227	F:5- GGATCGGTGAGTACCAACT-3 R:5- TTTCCTCCTCGGCAATAA-3	4	0.7	176	58	(TGTTT) ⁷
SSR22071	F:5- GCTGCTTGAATCGGTTCTGT-3 R:5- GAGGAGTAAATCATGCTCCA-3	6	0.72	204	58	(AAG) ⁸
SSR05737	F:5- TTGCTTCGTAAAGCAAAA-3 R:5- GAAGTAAATGGTTGGAC-3	5	0.66	185	54	(CT) ⁷
SSR19430	F:5- TATGGCGAAGCTTTGCT-3 R:5- AGGGGATCTTGCCTCTA-3	7	0.76	190	52.7	(AAG) ¹¹
SSR05125	F:5- TGGGAAAAAGGTATGGAAA-3 R:5- TGGCTCCTTCACATTGTTGT-3	4	0.64	220	57	(GAAA) ⁷
SSR06660	F:5- GATCGTTGCAAAACTCACGA-3 R:5- CGATTGACAGTTCGCTGAAA-3	2	0.77	197	54	(AG) ¹⁴
SSR16842	F:5- TGGGGTTAGGGTTCAAGTG-3 R:5- CAAGCTCTTCTCAACGGAC-3	5	0.78	144	58.1	(AGA) ¹¹
SSR13251	F:5- GGTCATCCAAAAGAGAAAGCA-3 R:5- ATCAACACCATGACGACCA-3	7	0.8	162	54	(AAG) ¹⁴
SSR15477	F:5- CTGCCAATTCGGTTTGAT-3 R:5- AATTCCTTGGGAAATGGCCT-3	2	0.44	213	54	(AAA) ¹²

Molecular analysis

The results of the polymerase chain reaction were separated on electrophoresis gels (Figure 2, 3 and 4). In total, 41 alleles in the studied genotypes were amplified with a mean of 4.55 per markers. The highest number of alleles was produced by primer SSR19430 (7 alleles) and the lowest numbers of alleles were created by primers SSR06660 and SSR15477 (2 alleles). The size of the bands was between 144 (SSR16842) and 220

(SSR05125). The mean PIC was 0.69. The highest amount of PIC in a marker (SSR13251) was 0.8 and the lowest (SSR15477) was 0.44. The high level of PIC in this study is indicative of the high level of differentiation. The amount of polymorphic information is an important parameter to compare markers, in terms of differentiation power. The high amounts of this criterion indicates high polymorphism and the presence of a rare allele or alleles at one marker locus and shows the high

Table 3. Measured morphological traits of 20 cucumber genotypes

No.	Trait	Mean	SD	Minimum	Maximum	Change range
1	plumule length	2.57	0.77	1	4	3
2	root length	5.87	1.18	4	7.90	3.9
3	number of primary branches per plant	2.33	0.49	2	3	1
4	days remaining to beginning of flowering	81.57	4.62	70	92	22
5	number of flowers per plant	30.22	8.75	20	50	30
6	days remaining to 3-leaf stage	44.01	6.03	32	51	19
7	days remaining to 4-leaf stage	52.66	5.64	40	62	22
8	days remaining to 5-leaf stage	61.90	6.81	51	72	19
9	days remaining to 6-leaf stage	69.55	7.26	51	80	29
10	plant height	22.56	6.38	12	35	23

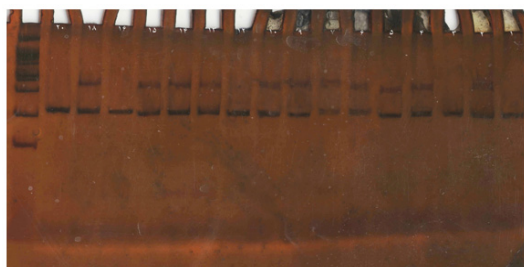


Fig. 2. Electrophoresis gel of PCR product of cucumber genotypes with primers SSR05125. The size of used marker 100 bp



Fig. 3. Electrophoresis gel of PCR products of cucumber genotypes with primer SSR12227. Size of marker was 100 bp

discriminatory and differentiation power of that marker (Varshney *et al.*, 2004)

Cluster analysis based on SSR markers

The Mantel test (Mantel, 1967) for the Jaccard similarity coefficient, Dice and simple adaptation based on UPGMA method was performed. According to the Jaccard similarity coefficient matrix, the coefficient of genetic similarity among the studied genotypes ranged from 0.51 to 0.92. Genotypes 2 and 3 of the Markazi and West Azarbajejan provinces, respectively, showed the highest similarity among

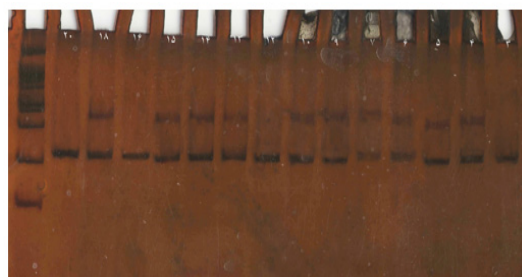


Fig. 4. Electrophoresis gel of PCR product with primer SSR15477. Size of marker was 100 bp

Table 4. Mean of squared analysis of variance of measured traits in cucumber genotype

Source of variation	Degree of freedom	Plumule length	Root length	Number of primary branches	days remaining to beginning of flowering	number of flowers per plant	Days to 3-leaf stage	Days to 4-leaf stage	Days to 5-leaf stage	Days to 6-leaf stage	Plant height
replication	2	0.07	0.17	0.03	3.12	9.38	6.35	2.38	0.79	6.16	2.1
Genotype	19	1.68**	4.24**	0.39*	58.81*	233.41*	105.97**	90**	136.22**	155.41**	123.49**
error	38	0.09	0.039	0.17	3.79	2.25	3.48	4.44	4.2	4.14	1.74
Coefficient variation (cv)		11.91	3.36	18.1	2.38	4.96	4.24	4	3.31	2.92	5.85

*, **: respectively significant in possible level of 1 to 5 percents

the studied genotypes with a genetic similarity coefficient of 0.94. Genotypes 6 and 12 of the provinces of Hormozgan and West Azarbayegan (Uromieh), respectively, showed the lowest similarity among studied genotypes with a genetic similarity coefficient of 0.51. The cluster analysis of the studied cucumber genotypes using UPGMA (Figure 5) resulted in three groups. The first group included genotypes 1, 2, 3, 4, 9, 10, 11, 12 and 13, among which 10, 11, 12 and 13 showed the greatest similarity among the genotypes of this category. Genotype 1 showed the most difference with the other genotypes in this category. The second group included genotypes 14, 15, 16, 17, 18, 19 and 20, among which 14, 15, 16 and 17 showed the greatest similarity among the genotypes of this category. The third group included genotypes 5, 6, 7 and 8, among which genotypes 5 and 6 showed the most similarities among the genotypes of this group. Genotype 8 showed the greatest difference with the rest of the genotypes in this category. In the principal coordinate analysis diagram, (Figure 6) genotypes 14, 15, 16, 17, 18, 19 and 20 are placed in a position relatively close to each other. Genotype 12 was placed at a farther distance and genotypes 11 and 13 were separated from each other and the other genotypes. Thus, the classification resulting from the principal coordinate analysis nearly corresponded to the cluster analysis classification. To show the correlation between the input matrix (molecular data) and the output matrix, the cophenetic correlation coefficient calculated for the molecular data was 0.66, showing that there was not a high correlation between the data.

DISCUSSION

Comparison of morphological and molecular data

The comparison of the cluster analysis, based the ten morphological characteristics, and SSR molecular data revealed that there was little similarity between these two patterns. The results of the principal components analysis confirmed the results of the cluster analysis. Moreover, the calculation of the cophenetic correlation coefficient (Mantel, 1967) between the matrix of similarity of morphological and molecular data ($r=0.25$) showed no significant correlation between them. As the heritability of morphological traits is very low

and environmental factors strongly influence these traits, the poor match of these traits with molecular data is realistic. In addition, there is a high accuracy and specificity of microsatellite markers and the heritability of these markers. However, since the number of markers used in this study is very low compared with the total genome, only a small part of the genome has been investigated and evaluated by microsatellite markers. Therefore, the possibility that these particular markers did

not cover the coding genes of morphological traits is also very high, resulting in the poor match between molecular data and morphological traits. This same discrepancy was found in a study of the genetic diversity of cucumber varieties in India (Pandey *et al.*, 2013). The results of this study demonstrated the ability of SSR molecular markers to separate different genotypes of cucumber. The results of a cluster analysis showed that geographic distances are not a reason for the genetic farness or

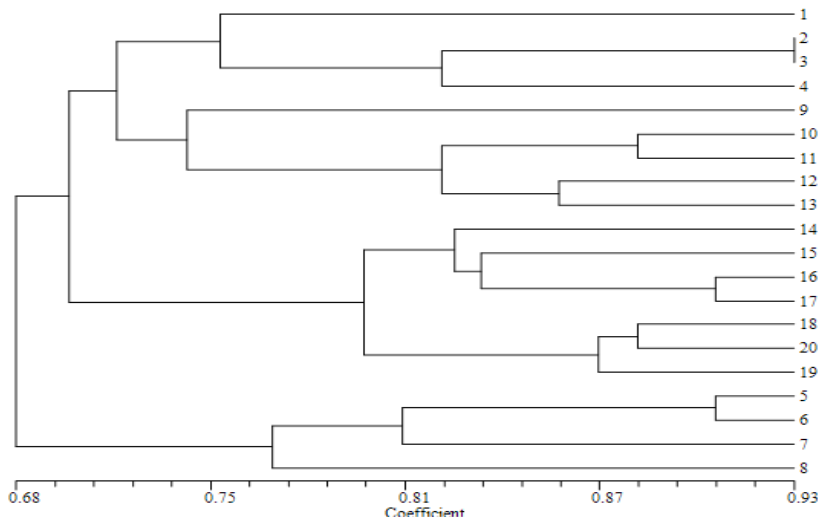


Fig. 5. Dendrogram of the cluster analysis of cucumber genotypes (*Cucumis sativus*) based on SSR data

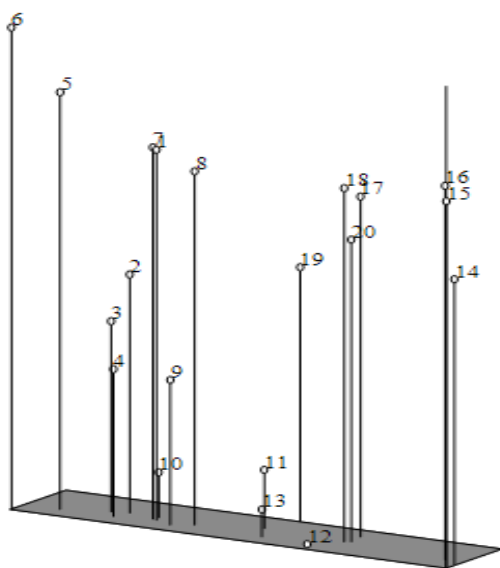


Fig. 6. Diagram of the distribution of 20 cucumber genotypes in the principal coordinate analysis

proximity of the studied genotypes. The analysis of SSR data suggests that, among the microsatellite markers used in this study, makers SSR16842 and SSR13251 (concerning the number of tracked alleles and the above polymorphic information content) are suitable for studying cucumber genotypes in Iran.

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