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EFFICACY OF ISSR AND SRAP TECHNIQUES FOR MOLECULAR CHARACTERIZATION OF SOME *CUCURBITA* GENOTYPES INCLUDING NAKED (HULL-LESS) SEED PUMPKIN

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ABSTRACT

This study was carried out to determine the efficacy of inter simple sequence repeats (ISSR) and sequence-related amplified polymorphism (SRAP) techniques for molecular characterization some *Cucurbita* genotypes including naked (hull-less) seed pumpkin. Plant material consisted of sixteen genotypes belonging to *Cucurbita pepo* L., and four genotypes belonging to *Cucurbita moschata* Duchesne, and four genotypes belonging to *Cucurbita maxima* Duchesne. A larger part of the *Cucurbita pepo* genotypes (7 Turkish and 4 foreign) were seed pumpkin and some of them were naked seed types used as appetizer and/or for oil production. Morphological characterization was also done according to UPOV criteria. The sixty bands were obtained by using 8 ISSR primers and all these bands were found polymorphic. In the SRAP study, 8 primer combinations were used, a total of 71 bands were scored and all these bands were polymorphic. In the ISSR analyses, the genetic similarity coefficients varied between 0.07 and 0.96, while in SRAP it was between 0.13 – 1.0. The correlation coefficient between ISSR and SRAP genetic similarity data was very high (r = 0.947). The genetic variation as measured by ISSR and SRAP markers revealed high diversity among Cucurbita genotypes (H = 0.30, I = 0.46 and 100 % polym.). The genetic diversity among Turkish naked seed pumpkin genotypes (H = 0.09, I = 0.14 and 26.7 % polym.) was only a little less than that of the foreign accessions (H = 0.12, I = 0.18 and 32.1 % polym.). Some differences were observed between molecular and morphological studies, and it was concluded that only morphological or molecular analyses but a combination of both approaches are often more reliable in genetic variability studies in Cucurbita genus.

Keywords: Pumpkin, Squash, Genetic relationship, SRAP, ISSR.

INTRODUCTION

Cucurbita genus belongs to Cucurbitaceae family and comprises of five domesticated species; *C. pepo* L. (Summer squash and Zucchini), *C. maxima* Duchesne (Pumpkin), *C. moschata* Duchesne (Butternut), *C. argyrosperma* Huber (*C. mixta* Pang.) (Cushaw) and *C. ficifolia* Bouché and all being native to America (Hadia *et al.* 2008). Turkey is not in the primary center of diversification area of the Cucurbita genus. However, it is an important squash and pumpkin producer (379 000 tones of production in 22 000 ha area in 2009 (FAOSTAT 2010) and has a great genetic diversity in the cultivated forms of this crop. *Cucurbita pepo* L. is one of the most variable species in the plant kingdom (Dushesne (1786): from Paris *et al.* (2003); Naudin (1869: from Ferriol *et al*. (2003 a, b). Three kinds of this fruit include pumpkins for edible round-fruited sorts, where as squash is the edible non-round-fruited sorts and gourds are the non-edible sorts (Paris *et al.* 2003). In Turkey pumpkins and squashes are mostly cultivated for culinary and snack purposes.

Cucurbita pepo seeds normally have thick, leathery seed coat (hull) due to the strong lignifications of some of its testa layers. The mutant Styrian (Naked (Hull-Less) seed pumpkin (*Cucurbita pepo* L. convar. *pepo* var. *styriaca* Greb. Winkler (2000)) exhibits a complete lack of lignifications of the testa. The proto-chlorophyll content of the fifth testa, chlorenchym layer, gives the seeds an olive-green color. This mutation emerged probably in the 1880's in the South-East of the then Austro-Hungarian-Monarchy (Zraidi *et al.* 2003). Idouraine *et al.* (1996) stated that naked seeds having the advantage of lacking the seed coat makes them cost effective to produce since there is no need for the expensive decorticating process and are therefore, favored by the oil and nut industries for commercial production.

There have been some studies about the morphological diversity among the landraces of the squash from different centers of diversity such as Cuba, Korea and Puerto Rico and as a result of these researches it has been revealed that there is a great variability of the squash types (Rios *et al.* (1997); Chung *et al.* (1998); Wessel-Beaver (1998)). Recently, Ferriol *et al.* (2004 a,

b) also found a considerable morphological variability among different landraces of *C. moschata* and *C. maxima*, especially in the fruit shape, ribbing and size. Morphological features are traditionally used to assess genetic variation in Cucurbita species. However, many cases are controlled by quantitative factors and/or affected by environmental modification (Dijkhuizen *et al.* 1996). In addition to morphological analysis many studies on molecular aspects have been undertake using different molecular markers such as RFLP, RAPD, SRAP, ISSR and AFLP to study genetic variation within and among several species of *Cucurbita* (Torres-Ruiz and Hemleben (1991); Young and Chung (1998); Katzir *et al.* (1998); Katzir *et al.* (2000); Baranek *et al.* (2000); Gwanama *et al.* (2000); Paris *et al.* (2002); Montes-Hernandez and Eguiarte (2002); Ferriol *et al.* (2003 a, b); Ferriol *et al.* (2004 a, b); Esteras *et al.* (2008); Tsivelikas *et al.* (2009)). Among different molecular markers, some are relatively cheaper, faster, reliable and simple to utilize in variety of applications. Two of such useful markers system have been employed in this study: (i) SRAP markers (Li and Quiros 2001), which preferentially amplify open reading frames and have been applied successfully in diversity analyses of *C. maxima* and *C. pepo* (Ferriol *et al.* 2003b; 2004a) and (ii) ISSR markers (Zietkiewicz *et al.* 1994), are based on the amplification of DNA regions located between two microsatellites loci and used successfully in diversity analyses of *C. pepo* (Katzir *et al.* (1998); Katzir *et al.* (2000); Paris *et al.* (2003)).

The objective of this study was to identify the polymorphisms and determine genetic relationships among some *Cucurbita pepo* genotypes collected in Turkey grown mostly for seed production including naked (hull-less) seed accessions. The parameters studied included genetic relatedness among shelled and naked seed pumpkin genotypes originated from Turkey compared to shelled and naked local and foreign pumpkin and squash genotypes.

MATERIALS AND METHODS

Plant material: Plant material consisted of twenty-four genotypes which belong to three species: *Cucurbita pepo* L.*,* *Cucurbita moschata* Duchesne, and *Cucurbita maxima* Duchesne (Table 1; Fig. 1). Five of the genotypes (Col. no. 5, 7, 10, 16 and 23) were from naked seeds of pumpkins, which were selected by Abak *et al.* (1990) at Cukurova University, Adana-Turkey..

Morphological Characterization: Seeds from 24 accessions were germinated in a greenhouse (Table 1) in March 2007. Ten plants per accession were transplanted 15 days later in an open field of the Cukurova University, with a plant spacing of 2 x 1 m. Ten plants per accession were evaluated for morphological characteristics, based on UPOV descriptor for *Cucurbitaceae* (some traits based on Duzeltir (2004). Fifty-one morphological characters were assessed, starting from young seedling until the stage of mature fruits and the extraction of the seed (Table 2).

Genomic DNA Extraction: Genomic DNA was extracted from young leaf tissues (three weeks old) for each of the genotype. Leaves were homogenized using liquid nitrogen and genomic DNA was extracted by CTAB method according to Kafkas *et al.* (2005).

ISSR-PCR analysis: ISSR primers 807, 809, 810, 812, 841, 842, 854, and 855 from Kit #9, University of British Columbia, Canada, were applied (Katzir *et al.* (1998); Katzir *et al*. (2000); Paris *et al.* (2003), Table 3). ISSR reaction mixture and protocols were as described by Katzir *et al*. (2000). Standardized PCR reaction mixture consisted 10 ng genomic DNA, 5X PCR buffer (Promega), 1.5 mM of $MgCl_2$ (Promega), 100 μM of dNTPs (Promega), 0.3 μM of primer and 1 unit of *Taq* polymerase in a 25 μl volume. Thermal profile for amplification was: 7 min for denaturing at 94 °C, at 35 cycles with three steps for each one: 1 min of denaturing at 94 °C, 1 min of annealing at 50-54°C (depended on primers), and 2 min of elongation at 72°C. The reactions were followed by a 7 min extension at 72°C and eventually stored at 4 °C. The amplified ISSR products were separate by electrophoresis on 1.8 % agarose gel with 0.5x TBE buffer. After staining with ethidium bromide, banding patterns were visualized with a UV transilluminator.

SRAP analysis: In this assay, 8 different combinations were employed using five forward primers and five reverse primers (Table 3). SRAP reaction mixture and protocols were used as described by Ferriol *et al.* (2003). Each 25 μl PCR reaction mixture consisted of 20 ng genomic DNA, 200 μM of dNTPs, 1.5 mM of $MgCl_2$, 0.3 μM of primer, 10 x *Taq* buffer and 1 unit of *Taq* polymerase (Fermantes). Samples were subjected to the following thermal profile for amplification in an oven thermocycler (Eppendorf Mastercycler Gradient): 5 min of denaturing at 94°C, five cycles of three steps: 1 min of denaturing at 94°C, 1 min of annealing at 35°C and 2 min of elongation at 72°C. In the following 30 cycles the annealing temperature was increased to 50°C, with a final elongation step of 5 min at 72°C. The amplified SRAP products were separate by electrophoresis on 2 % agarose gel with 0.5x TBE buffer. After staining with ethidium bromide, banding patterns were visualized with a UV transilluminator.

Data analysis: Twenty-four Cucurbita accessions were investigated for morphological, taxonomic relationships; the data were also obtained to determine their molecular and genetic distance. Polymorphisms detected at 60 loci by using 8 ISSR primers, 71 loci by using 8 SRAP primer pairs were used in the genetic evaluation of 24 pumpkin genotypes (Table 4, 5).

The molecular genetic diversity among squash genotypes was determined by using Jaccard (J) distance matrix (Sokal and Sneath 1963). A presence(1)/absence(0) binary data matrix obtained from scoring polymorphic inter simple sequence repeats (ISSR) and sequence-related amplified polymorphism (SRAP) bands was used to calculate J similarity coefficients to estimate the molecular genetic diversity among squash genotypes. The UPGMA cluster analysis, the resulting dendrogram and multidimensional scalings (MDS) were performed on the J genetic distance matrix using the computer program NTSYpc version 2.02k (Rohlf 1997). The morphological genetic diversity among melon genotypes was determined by using Nei–Li distance measure (Nei and Li 1979). Whether the degree of morphological difference between samples is related to the molecular distances was tested by the use of the Mantel test (Mantel 1967). MDS produces a statistic called *stress* indicating a goodness of fit of the distances in the configuration space to the monotone transformation function of the original distances. *Stress* values vary between 0 and 1 (0.40: poor; 0.20: fair; 0.10: good; 0.00: perfect goodness of fit) (Kruskal (1964); Rohlf (1997)). In the dendrogram (Fig. 3) and MDS scaling (Fig. 4) distinct genotypes of are depicted in different colors.

The computer program POPGENE (Yeh *et al.* 1997) was used to calculate the statistical measures of genetic variation (i.e., Nei's gene diversity (Nei 1973), Shannon's information index (Shannon and Weaver 1949) and percentage of polymorphic loci) as measured by molecular markers for Cucurbita genotypes.

RESULTS AND DISCUSSION

Characterization of different accessions exhibited considerable diversity for most of the morphological characters evaluated. The UPGMA method, based on "Nei-Li distances", grouped the accessions according to the dendrogram of Fig. 2. Cucurbita accessions were divided into two main groups, one of them was large and the other was small. The naked seeded *C. pepo* accessions (5, 6, 8, 9, 10, 12, 13, 14, and 15) and one seeded *C. pepo* accession (11) tended to be grouped. On the other hand, *C. maxima* and *C. moschata* accessions were not clearly discriminated from the other *C. pepo* accessions (Fig. 2).

In the ISSR study, 60 bands were obtained by using 8 ISSR primers and all these bands were found polymorphic (Table 4). The number of fragments detected by an individual primer ranged from 4 (UBC 810 and UBC 824) to 15 (UBC 809), with an average of 7.5. Rp values ranged from 0.48 (UBC 812) to 1.13 (UBC 854) with a total of 6.6. The PIC values of the primer ranged from 0.60 (UBC 809) to 0.90 (UBC 812) with an average of 0.73 (4). In the SRAP study, 8 primer combinations were used, a total of 71 bands were scored and all these bands were polymorphic (Table 5). The number of fragments detected by an individual primer combination ranged from 3 (Me-2/Em-1) to 19 (Me-2/Em-6), with an average of 8.88. Rp values ranged from 0.44 (Me-1/Em-2) to 1.05 (Me-2/Em-3) with a total of 6.01. The PIC values of the primer combinations ranged from 0.65 (Me-2/Em-3) to 4.59 (Me-1/Em-2) with an average of 1.27 (Table 5). In the ISSR analyses, the genetic similarity coefficients varied between 0.07 and 0.96, while in SRAP it was between 0.13 – 1.0. The correlation coefficient between ISSR and SRAP genetic similarity data was very high (r = 0.947). The UPGMA method, based on "J distances", grouped the accessions according to the combined molecular ISSR and SRAP dendrogram (Fig. 3). Based on the combined molecular ISSR and SRAP analysis (Fig. 3), the most similar genotypes were the accessions 5 and 6 (0.93 J similarity coefficient) followed by the accessions 6 and 8 (0.90 J similarity coefficient), and by the accessions 6 and 10 (0.89 J similarity coefficient); the most dissimilar ones were the accessions 11-20, 13-20, 15-20, 20-24, and 13-27 (0.13 J similarity coefficient) (Fig. 3). Of all evaluated accessions, the most distinct ones were 20, 25, 27, and 30 (*C. maxima* and *C. moschata*) while the least distinct ones were 5, 6, and 10 (*C. pepo*). Cucurbita accessions were divided into two major groups based on the combined molecular ISSR and SRAP dendrogram, 2D scaling (Fig. 3-4). The *stress* values for all evaluated GD matrices in MDS scalings was 0.109, indicating good fit of the data. The accessions belonging to *C. pepo* (5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 22, 23 and 24) were in the main group while the accessions belonging to *C. maxima* (25, 30, 32, and 36) and *C. moschata* (20, 21, 27, and 28) were in two separate sub-groups in the second group (Fig. 3-4).

The statistical variation measures showed that the genetic diversities among Cucurbita accessions were significantly different (Table 6). The genetic diversity in all accessions was high. The genetic diversity in Turkish naked seeded *C. pepo* accessions was lower (I=0.136 vs. I=0.178) to that of foreign naked seeded *C. pepo* accessions.

Being in a temperate climatic zone, Turkey is rich in plant diversity because of its unique geomorphology, topography and climate. Cultural conditions and traditional agriculture practices preserved in several rural parts of the country have preserved a great deal of genetic diversity in all cucurbits (Sensoy *et al.* (2007); Sari *et al.* (2008)).

In the present study, different species and genotypes of squash and pumpkin including naked seed pumpkin were separated from each other based on characterization both with morphological characteristics as well as molecular (ISSR and SRAP) analysis methods. As a result of molecular evaluations, it was determined that *C. maxima* and *C. moschata* species was closer to each other, but *C. pepo* was included in a different group. The local inbred naked seed pumpkin lines obtained by selection in *C. pepo* were too close to each other genetically. High rate of genetic similarity among these lines was an expected result because these lines were selected with selection from the same population.

Table 1. Classification and sources of 24 Cucurbita accessions

Col. Number	Accession code (Cultivar name or local name)	Collection site	*Cucurbita* species	Seed properties
5	NO. 5	CU*	*C. pepo*	Naked seed
6	NO. 7	CU	*C. pepo*	Naked seed
8	NO. 10	CU	*C. pepo*	Naked seed
9	NO. 16	CU	*C. pepo*	Naked seed
10	NO.23	CU	*C. pepo*	Naked seed
11	22x23 F_1 Melez	CU	*C. pepo*	Seed pumpkin
12	Lady Godiva	Switzerland	*C. pepo*	Naked seed
13	Baby Bear	Germany	*C. pepo*	Naked seed
14	Gleisdorfer Ölkürbis	Austria	*C. pepo*	Naked seed
15	Foreign green	CU	*C. pepo*	Naked seed
16	Nevşehir 1	Nevsehir	*C. pepo*	Seed pumpkin
18	Nevşehir 3	Nevsehir	*C. pepo*	Seed pumpkin
19	Bolu Kabuksuz	OMU**	*C. pepo*	Naked seed
20	Iskenderun 2	Iskenderun	*C.moschata*	Seed pumpkin
21	Iskenderun 3	Iskenderun	*C.moschata*	Seed pumpkin
22	Bolu Kabuksuz	OMU	*C. pepo*	Naked seed
24	Sakız 5801	Bursa	*C. pepo*	Zucchini
25	Bal kabağı	Konya	*C.maxima*	Winter pumpkin
27	55 B 02 Bal kabağı	OMU	*C.moschata*	Winter pumpkin
28	05 AM 02 Bal kabağı	OMU	*C.moschata*	Winter pumpkin
29	14 BO 01	OMU	*C. pepo*	
30	05 AM 14 Kestane kabağı	OMU	*C.maxima*	Winter pumpkin
32	05 AM 05 Kestane kabağı	OMU	*C.maxima*	Winter pumpkin
36	55 B 06 Bal kabağı	OMU	*C.maxima*	Winter pumpkin

*CU: Cukurova University, **OMU: Ondokuz Mayis University

Table 2. Fifty-one morphological characters used in the study.

cotyledon shape
cotyledon color
cotyledon width
cotyledon length
growth habit
branching
branching level
attitude of petiole
main stem color
main stem internode number
tendrils
leaf blade size
leaf blade incisions
leaf blade color
leaf blade blistering
leaf blade marbling
petiole color
petiole length
petiole diameter
petiole vertical cut shape
petiole surface spines number
female flower petal ring presence
female flower petal ring color
female flower petal ring color intensity
female flower sepal length
female flower pistil color before anthesis
male flower petal ring color intensity
male flower peduncle length
male flower peduncle diameter
male flower peduncle color
grooving at male flower peduncle
pubescence at male flower peduncle
male flower sepal length
fruit shape
fruit main color
fruit main color intensity
number of fruit skin color
secondary skin color
fruit diameter
fruit length
fruit length/diameter ratio
fruit grooves
color of grooves
main color of flesh
seed size
seed shape
seed cross-section shape
seed hilum end
seed coat color
seed coat thickness
seed coat removing

Table 3. Primer sequence used for ISSR and SRAP analysis

ISSR primer	Sequence (5' – 3')	Annealing Tempt. (°C)	SRAP primer	Sequence (5' – 3')
UBC 807	aga gag aga gag aga gt	50	Me-1 (forward)	tga gtc caa acc gga ta
UBC 809	aga gag aga gag aga gg	52	Me-2 (forward)	tga gtc caa acc gga gc
UBC 810	gag aga gag aga gag at	50	Me-6 (forward)	tga gtc caa acc gga ca
UBC 812	gag aga gag aga gag ac	50	Me-7 (forward)	tga gtc caa acc gga cg
UBC 841	gag aga gag aga gag atc	54	Me-8 (forward)	tga gtc caa acc gga ct
UBC 842	gag aga gag aga gag at	54	Em-1 (reverse)	gac tgc gta cga att aat
UBC 854	tct ctc tct ctc tct crg	54	Em-2 (reverse)	gac tgc gta cga att tgc
UBC 855	aca cac aca cac aca cyt	52	Em-3 (reverse)	gac tgc gta cga att gac
			Em-5 (reverse)	gac tgc gta cga att aac
			Em-6 (reverse)	gac tgc gta cga att gca

Tablo 4. The number of bands generated, polymorphism percentage and gene diversity estimates in all accessions as revealed by ISSR

Primer	Total fragment	Pol[a]	% pol[b]	Rp[c]	PIC[d]
UBC 807	6	6	100	0.72	0.83
UBC 809	15	15	100	0.60	0.60
UBC 810	4	4	100	0.90	0.74
UBC 812	5	5	100	0.48	0.90
UBC 841	10	10	100	1.00	0.68
UBC 842	7	7	100	0.95	0.69
UBC 854	4	4	100	1.13	0.62
UBC 855	9	9	100	0.88	0.73
Total	60	60	-	6.6	-
Average	7.5	7.5	100	-	0.73

[a] Number of polymorphic fragments, [b] Percentage of polymorphic fragments, [c] Resolving power, [d] Polymorphism information content

Table 5. Number of total and polymorphic fragments and gene diversity estimates in 24 accessions using SRAP

Combination	Total fragments	Pol[a]	% pol[a]	Rp[c]	PIC[d]
Me-1/Em-2	8	8	100	0.44	4.59
Me-2/Em-1	3	3	100	0.92	0.67
Me-2/Em-3	8	8	100	1.05	0.65
Me-2/Em-6	19	19	100	0.77	0.80
Me-6/Em-5	13	13	100	0.70	0.82
Me-6/Em-6	8	8	100	0.53	0.92
Me-7/Em-6	4	4	100	0.75	0.80
Me-8/Em-3	8	8	100	0.85	0.91
Total	71	71	-	6.01	
Average	8.88	8.88	100	-	1.27

[a] Number of polymorphic fragments, [b] Percentage of polymorphic fragments, [c] Resolving power, [d] Polymorphism information content

Table 6. Statistical measures of genetic variation as measured by ISSR and SRAP markers for 24 Cucurbita accessions

Cucurbita accesions	N[a]	H[b]	I[c]	% Polymorphism[d]
All genotypes	24	0.302	0.464	100.00
Turkish naked seed (*Cucurbita pepo*)	7	0.090	0.136	26.72
Foreign naked seed (*Cucurbita pepo*)	4	0.120	0.178	32.06
Seed (*Cucurbita pepo*)	5	0.129	0.191	34.35
Cucurbita maxima	4	0.082	0.126	25.19
Cucurbita moschata	4	0.137	0.206	38.17

[a]N= Number of genotypes in each population; [b]H= Nei's gene diversity;
[c]I= Shannon's information index; [d]: Percentage of polymorphic loci

Fig. 1. The photos of twenty-four genotypes investigated in the study

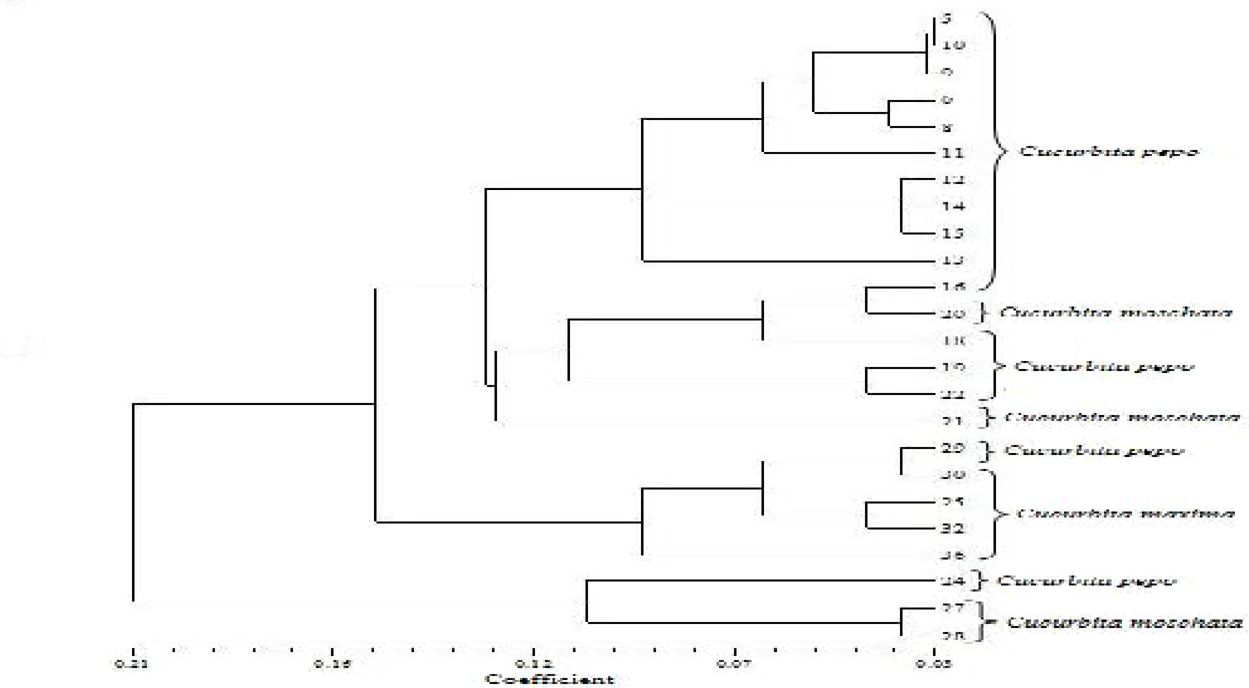

Fig. 2. Dendrogram showing the clustering of 24 accessions based on morphological data

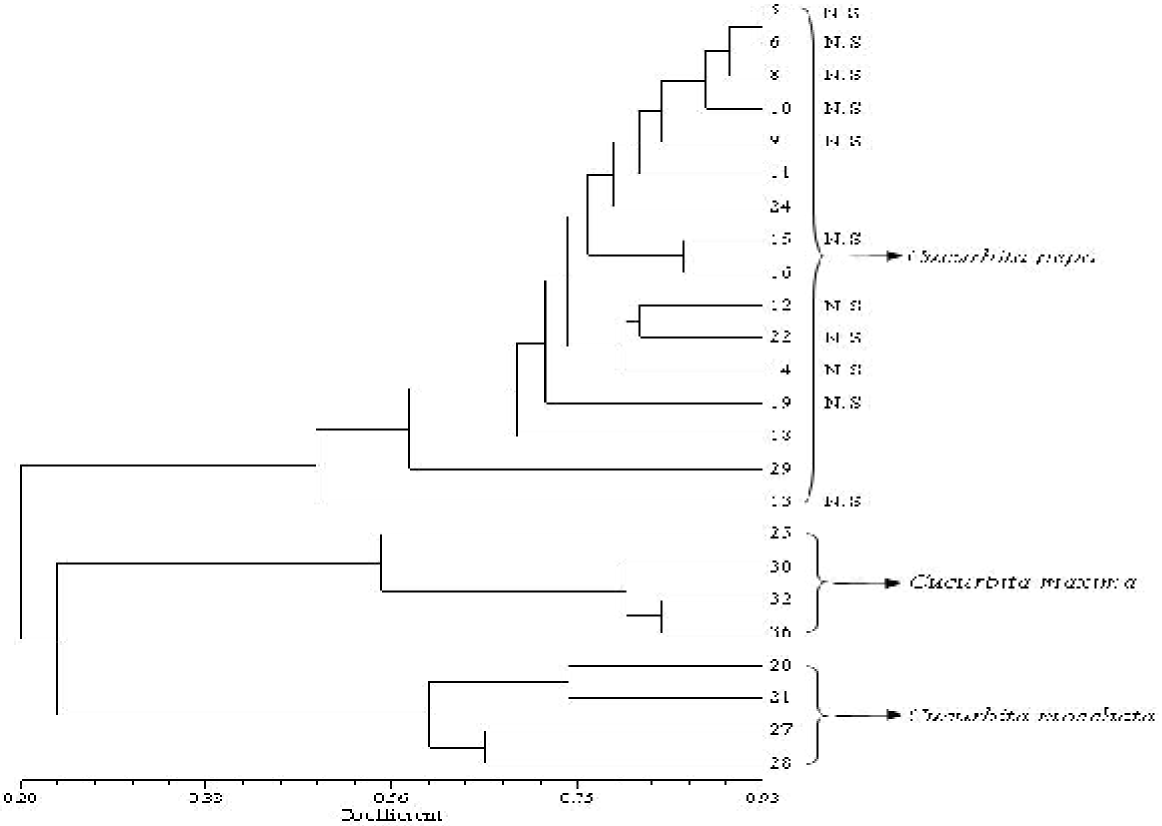

Figure 3. Consensus tree for twenty-four Cucurbita genotypes developed on the basis of their banding patterns with combination of ISSR and SRAP (N.S.= Naked Seed)

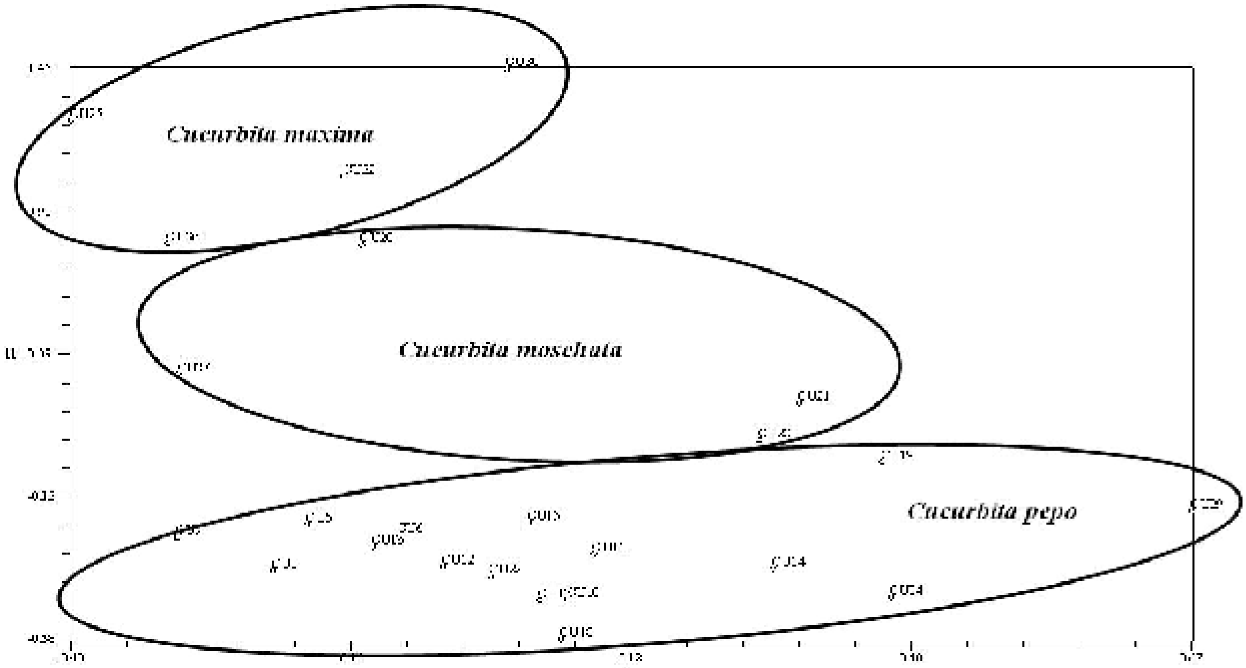

Fig. 4. Associations among Cucurbita accessions revealed by 2D scaling analysis.

The naked (hull-less) seed trait has been beneficial for the development of oil-seed pumpkins and pumpkin snack seed (Winkler 2000). There have been limited studies on naked seed pumpkin. Idouraine *et al.* (1996) studied the nutrients constituents from eight lines of Austrian naked seed *Cucurbita pepo* L. and revealed some significant differences on chemical and nutritional values. Nakic *et al.* (2006) evaluated the chemical characteristics of oils from naked and husk seeds of *Cucurbita pepo* L. and discovered some differences for fatty acid composition, tocopherol, sterol, and squalene content.

The naked seed pumpkin accessions used in the present study were the lines obtained from local selection of genotypes, and some originated in foreign. The findings indicated that the local and foreign naked seed pumpkin accessions used in the study were in the similar groups in the dendrogram, and hence closer to each other, It was therefore suggested that these Turkish and Central European naked seed pumpkin accessions might have come from the same ancestor. The study however, could not separate the naked seed and normal hull-seed pumpkins entirely due to the fact that there were few genes responsible for naked seed trait.

Although there were previously conducted genetic and histological studies on this topic, genetic studies have been limited to the classical Mendelian genetics; moreover, contradictory information in the literature on this subject took place (Zraidi *et al.* (2003); Stuart and Loy (1983)). Bezold *et al.* (2005) studied the molecular basis of hull-less trait in pumpkin, and stated that the expression patterns of several genes involved in secondary cell wall biosynthesis during the development of the testa. Additionally, the expression patterns of a few genes that were differentially expressed during testa development are presented by Bezold *et al.* (2005).

The genetic variation as measured by ISSR and SRAP markers in the present study revealed high level of diversity among Cucurbita genotypes (H = 0.30, I = 0.46 and 100 % polym.); the genetic diversity among Turkish naked seed pumpkin genotypes (H = 0.09, I = 0.14 and 26.7 % polym.) was only a little less than that of the foreign accessions (H = 0.12, I = 0.18 and 32.1 % polym.). Wu *et al.* (2011) studied the molecular diversity of Chinese *Cucurbita moschata* germplasm collections (Seventy-four Chinese accessions and 15 accessions from other countries) detected by Amplified Fragment Length Polymorphism (AFLP) markers (Nine pairs of EcoRI/MseI primers produced 500 fragments, of which 75.57% were polymorphic, indicating a high degree of diversity). The genetic variation estimates for four Turkish *C. moschata* genotypes (H = 0.13 and I = 0.21 were not much lower than those of genotypes WU et al. (2011) (H = 0.20 and I = 0.29). Ferriol *et al.* (2003a) studied the genetic diversity of 19 accessions of *Cucurbita maxima* along with 8 related *Cucurbita* accessions from Spain using Randomly Amplified Polymorphic DNA (RAPD) and Sequence Based Amplified Polymorphisms (SBAP) markers and found 57 % and 33 % polymorphisms for these markers, respectively. In the present study using ISSR and SRAP markers, we found 100 % polymorphisms for these markers, respectively.

Ferriol *et al.* (2003b) studied the genetic diversity of a germplasm collection of *Cucurbita pepo* (69 accessions) using SRAP and AFLP markers and stated that the information given by SRAP markers due to being sequence based was more concordant to the morphological variability and to the evolutionary history of the morphotypes than that of AFLP markers. These researchers found 72.5 % and 52.4 % polymorphisms among *Cucurbita pepo* using SRAP and AFLP markers, respectively.

Ferriol *et al.* (2004b) studied the morphological and molecular diversity of a collection of *Cucurbita maxima* landraces and determined that SRAP marker analysis grouped the accessions in accordance to their type of use and AFLP marker analysis grouped the accessions as to their geographical origin. Ferriol *et al.* (2004a) studied the genetic diversity of a germplasm collection of *Cucurbita moschata* (47 accessions) using SRAP and AFLP markers and detected that accessions clustered according to geographical origin with both markers. In the present study, the same set of SRAP primers were successfully applied to evaluate genetic diversity among the accessions and the correlation coefficient between ISSR and SRAP genetic similarity data was very high.

Some differences were also observed between molecular and morphological studies, and it was concluded that morphological analyses are only valid and reliable when coupled with molecular data for variability studies in Cucurbita genus. Molecular evaluation was more favorable than phenotypic evaluation because it had more markers and represented neutral traits of simple inheritance (Sensoy *et al.* 2007).

Conclusively assessment of genetic distance and diversity among different Cucurbita species including naked (hull-less) seed pumpkin reported here, could be useful in further genetic studies and the selection and improvement of the most adequate accessions for use in breeding programs. Moreover, the use of co-dominant molecular markers (Esteras *et al.* 2008) would be more informative in extensive future genetic variability studies in Turkish Cucurbita germplasm.

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