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Investigation of genetic diversity of squash (*Cucurbita maxima* Duchesne*)* genotypes collected from Erzincan in Turkey

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ABSTRACT: Plant genetic resources constitute the most valuable assets of countries. It is of great importance to determine the genetic variation among these resources and to use the data in breeding studies. *Cucurbita maxima* species in the Cucurbitaceae family have high genetic diversity, but molecular genetic diversity studies of this species are insufficient in Turkey. To determine the genetic diversity among genotypes of *Cucurbita maxima* species of squash, which is widely grown in Erzincan, 14 different squash genotypes collected were examined based on the morphological parameters and molecular characteristics. According to the evaluated morphological characteristics, we can say that genotype 5 should be evaluated in breeding programs. In addition, in crossbreeding studies, the heterosis feature is more successful in types with a long genetic distance. In the study, the longest genetic distance was found between genotypes 5 and 48. Simple sequence repeat (SSR) markers were used to determine genetic diversity at the molecular level. The analysis of morphological characterization within genotypes showed a wide variability in morphological traits of plant, flower, fruit, and leaf. Seven SSR markers yielded a total of 23 polymorphic bands, the number of alleles per marker ranged from 2 to 5, and the mean number of alleles was 3.286. Polymorphic information content (PIC) ranged from 0.00 (GMT-M61) to 0.202 (GMT-P25), and the mean PIC value per marker was 0.130. Cluster analysis using Nei's genetic distance determined that 14 genotypes were divided into 3 major groups.

KEY WORDS: Characterization, *Cucurbita maxima*, genetic diversity, squash, SSR markers.

INTRODUCTION

Turkey is one of the countries where different cultivated squash species are grown, including *Cucurbita maxima* Duchesne (Ferriol *et al.,* 2003). *Cucurbita maxima* Duch. (Winter squash, pumpkin) is a species belonging to the genus *Cucurbita*, and this species is both economically important and has high genetic diversity (Kaźmińska *et al.,* 2017). Pumpkin or winter squash fruits are a valuable source of nutrient content (sugars, fatty acids, fiber, carotenoids, vitamins and minerals, etc.) thus they are used as both human consumption and also for animal feeding. Due to their important effects on human nutrition and health, demand for pumpkin and winter squash fruits has increased in recent years and they have been used in the development of new types of food products (Konopacka *et al.,* 2010).

The *Cucurbita maxima* is characterized by high adaptability to different ecological conditions (Kaźmińska *et al.,* 2017). In addition to being the gene center of many plant species, Turkey has an important place in the world in terms of plant genetic diversity. However, Turkey also has very rich gene resources in terms of vegetable species as well as many plant species (Öztürk *et al.,* 2020). It is stated that Turkey has a great genetic diversity especially for mastic squash or pumpkin (Küçük *et al.,* 2012). The most common cultivated species of the squash or pumpkin in Turkey are *C. maxima*, *C. moschata*, and *C. pepo* (Balkaya *et al.,* 2009). Although Turkey is outside the area of primary genetic diversity for *Cucurbita* species, its geographical location and favorable ecological conditions have allowed *Cucurbita* species with significant genetic diversity over the years (Dalda-Şekerci *et al.,* 2020). Because, Turkey has become one of the important diversity centers for cucurbits cultivated as cucurbits (including Cucurbita maxima) adapt to different ecological conditions as a result of both natural selection and farmer selection (Balkaya *et al.,* 2010). In addition winter squash local species are sometimes grown as undeveloped populations in different regions of Turkey. Seed exchange among farmers could allow for the maintenance of genetic diversity in these winter squash populations. These traditional native breeds are an important genetic resource for plant breeders due to their significant genotypic variation. This variation is supported and maintained by the conscious selection for certain traits by the farmers (Balkaya *et al.,* 2009).

Determination of morphological properties is the first stage in the description and classification of plant genetic resources (Hamdi *et al.,* 2020). However, morphological identification studies alone are not sufficient. In order to obtain clearer results from these genetic diversity identification studies, it is necessary to use molecular markers. Genetic diversity studies using molecular markers are important for increasing precision and

precious cultivars by quicken the selection process, helping to determine the rate of variation, and selecting suitable parental in breeding programs (Kaźmińska 2016). However, despite the agricultural and biological importance of squash/pumpkin (*Cucurbita* spp.) species, molecular studies have been very limited so far. Today, the widespread use of biotechnological methods has provided many advantages in crop breeding. Different DNA markers have been used successfully in diversity studies evaluating inter- and intra-species genetic relationships. Many studies have been conducted to examine genetic diversity among Cucurbita species using various molecular markers such as AFLP (Ge *et al.,* 2015), RAPD (Ntuli *et al.,* 2015), ISSR (Dalda-Şekerci *et al.,* 2020), SRAP (Zheng *et al.,* 2016), and SSR (Nyabera *et al.,* 2021). Most marker systems used to date have limitations associated with their dominant and/or unreliable nature. Simple sequence repeats (SSRs) are suitable to detect variation within varieties since they are reliable, co-dominant and highly polymorphic as well as detect high levels of allelic diversity (Formisano *et al.,* 2012). After these markers were first found in humans (Litt and Luty, 1989), they began to be used in other living organisms as well. SSRs are repetitive DNA sequences of 1-6 base pair units (Queller *et al.,* 1993; Kashi *et al.,* 1997), with abundance abundant in the genome. Certain SSR markers have functional significance in chromatin organization, regulation of gene activity, and recombination (Li *et al.,* 2002), but they are more often apparently randomly distributed in the nonfunctional genomic regions. SSR markers can be used effectively in population genetics and gene mapping studies because of their advantages as an informative marker system including requiring small amounts of DNA, being codominant and stable, being abundant and scattered throughout the genome, being reproducible and suitable for automation, and having a high level of polymorphism (Powell *et al.,* 1996). SSR marker system were used to determine genetic variability within *Cucurbita maxima* Duch. species (Corazza-Nunes *et al.,* 2002; Ge *et al.,* 2015; Kaźmińska *et al.,* 2017; Nyabera *et al.,* 2021). The SSR technique has successfully been used in the assessment of genetic diversity in cucurbit species such as pumpkin/squash (Kaźmińska *et al.,* 2017; Kayak *et al.,* 2018; Duman *et al.,* 2020; Yunli *et al.,* 2020), snake melon (Merheb *et al.,* 2020) watermelon (Mujaju *et al.,* 2010; Mashilo *et al.,* 2017), bitter melon (Karaman *et al.,* 2018), cucumber (Dar *et al.,* 2017). The rate of foreign fertilization in squash is very high. Due to foreign pollination, lines different from the original seed may occur, leading an increased genetic variation. Over time, squash cultivars have spread to the regions of our country with both natural and artificial selections and have been formed from different populations in these regions (Dalda-Şekerci *et al.,* 2020). This type of plant genetic resources in our country establishes the basis of genetic materials of breeding studies. However, it is important to prevent the disappearance of such local genetic resources to be used in breeding studies. Despite its economic and nutritional importance, few molecular genetics studies have been conducted on *Cucurbita maxima.* A comprehensive characterization study consisting of morphological and molecular parameters in *Cucurbita* ssp. has not yet been carried out in Erzincan province.

Cucurbita maxima populations in Turkey show high variation in terms of morphological characteristics. However, this diversity has not been sufficiently characterized in Turkey. In addition, a detailed investigation of morphological and molecular variation in *C. maxima* populations was not conducted in Erzincan province where the study was conducted. In this study, it was aimed to determine the morphological characteristics of squash genotypes of *Cucurbita maxima* species, which is widely grown in Erzincan province. In addition, it is aimed to provide additional information for pumpkin breeding studies in Turkey by determining the genetic relationships between genotypes with the SSR marker method.

MATERIALS AND METHODS

Plant material

In this study, the 14 squash genotypes were collected from different regions of Erzincan province (Table 1; Figure 1). Seedlings of 14 different genotypes were produced in the unheated greenhouse of the Erzincan Horticultural Research Institute. Morphological and molecular identification studies of 14 local squash genotypes collected were performed.

Determination of morphological properties

Morphological identification studies were carried out in the fields and laboratories of the Erzincan Horticultural Research Institute. Genotypes were evaluated in terms of different phenotypic characteristics including plant (growth habit, branching, degree of branching), leaf (Position of the leaf stalk, leaf blade size, incisions, intensity of green color), petiole (attitude of petiole, green color, length, thickness, degree of prickles) and fruit (shape, major color, intensity of major color, number of colors, diameter, length, indices) traits. The morphological characters of the plants were determined by taking into account the standards set for *Cucurbita maxima* Duch by UPOV (International Union for the Conservation of New Plant Varieties) (UPOV, 2009).

SSR analysis

For SSR analysis, plant genomic DNA was isolated with minor modifications to the protocol defined by Saghai-Maroof *et al.,* (1984). 50 ml isolation buffer was prepared and heated to 70°C in a water bath and 100 µl of β-mercaptoethanol [Merck ®] was added into it. The

Table 1. Coordinate information of the regions where squash genotypes were collected.

Number	Genotype code	Site-Location	Altitude (m)	Latitude	Longitude
1	≠5	S1-Bahçeliköy village	1371	39°45'	39°20'
2	≠12	S2-Çatalarmut village	1443	39°48'	39°18'
3	≠15	S3-Çayırlı district	1547	39°50'	40°00'
4	≠16		1547	39°50'	40°00'
5	≠18		1290	39°41'	39°41'
6	≠20		1290	39°41'	39°41'
7	≠21		1290	39°41'	39°41'
8	≠22		1290	39°41'	39°41'
9	≠24		1290	39°41'	39°41'
10	≠43	S4-Cevizli village	1290	39°41'	39°41'
11	≠44		1290	39°41'	39°41'
12	≠45		1290	39°41'	39°41'
13	≠47		1400	39°43'	39°21'
14	≠48		1400	39°43'	39°21'

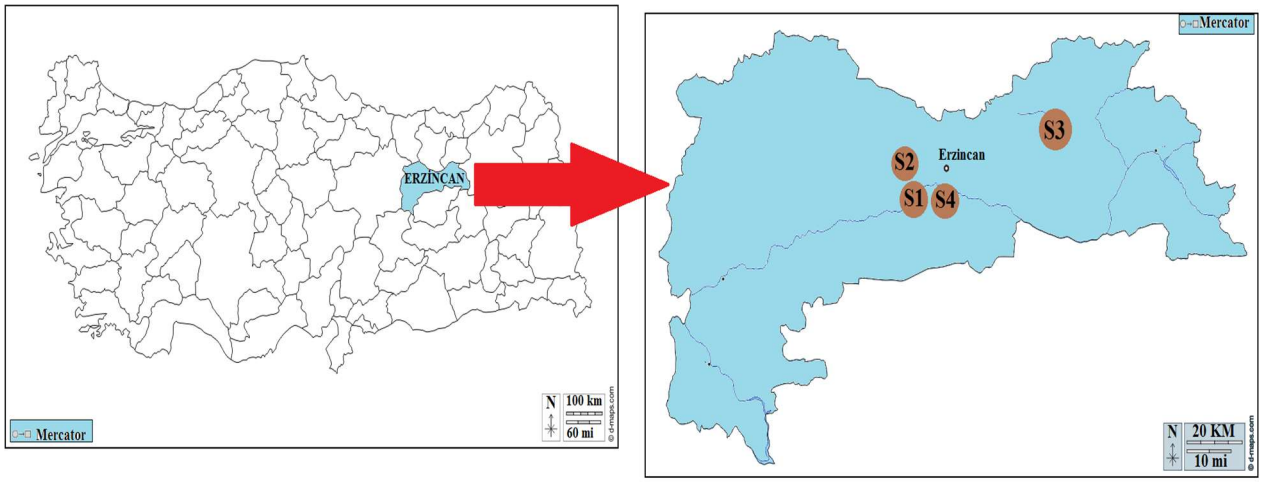

Fig. 1. Geographic distribution of ***Cucurbita maxima*** landraces collected from different geographical provinces of Erzincan, Türkiye (Table 1; S1: Bahçeliköy village, S2: Çatalarmut village, S3: Çayırlı district, S4: Cevizli village).

samples were weighed on a precision balance to 0.3 g and grinded with liquid nitrogen. The ground samples were taken into 2.0 ml eppendorf tubes, 1000 µl of isolation buffer solution was added, and incubated in a 70 ºC water bath for 60 minutes by turning upside down every 10 minutes. 750 µl of chloroform: isoamyl alcohol (24:1) was added to the samples and slightly turned upside down. Mixed samples were centrifuged at 14000 rpm for 20 min at 4 °C. At the top layer (supernatant) of the three layers formed was removed using a pipette and transferred to new eppendorf tubes. The same proportion of chloroform:isoamyl alcohol was added again to the supernatant and centrifuged at 14000 rpm for 20 min at 4 °C. The upper phase was transferred to new eppendorf tubes and 100 µl of 10 M ammonium acetate and 100 µl of 3 M sodium acetate were added. 2.5 times of isopropanol (-20 °C) was added to the resulting mixture and slightly turned upside down. When the DNA pellet was seen, the eppendorf tubes were centrifuged at 14000 rpm for 20 min at 4 °C. The supernatant was obtained by removing the liquid part from the tubes. The tubes were centrifuged at 14000 rpm for 1 min at 4 °C and then left to dry in the incubator at 37°C for 15 minutes. 100 µl of TE buffer was added to the genomic DNAs obtained from the samples and stored at +4 °C. To measure the purity of DNA samples, 4 µl of DNA + 996 µl of TE buffer was added, and absorbance (A) values were read in the spectrophotometer at 260 nm and 280 nm wavelengths. DNA samples with a 260/280 value between 1.1 and 1.8 were labeled as pure DNA. Using the formula 50 (multiplication coefficient for DNA) x 250 (dilution coefficient) x OD 260 (read value at 260 nm), the amount of DNA in the stock was calculated and working solutions containing 50 ng/l DNA were prepared from the stock DNA. Information about the SSR primers used in our study is given in Table 2.

Data analysis

Polymorphic bands of each SSR marker were scored for either their present (1) or absent (0). The binary matrix was constructed and used in statistical analyses. The PIC values of each SSR markers were calculated using the

Table 2 Information about the SSR primers

Primers	Repeat motif	Forward primer (3´-5´) Reverse primer (5´-3´)
CMTp18	$(TC)_{17}$	F: ACACCTTCGCTTCCGACATC R: TGACATCACTCCGGCAACTC
CMTm25	$(TTCTTCT)_5$	F: CTGACGTCGCTACTCATAGCA R: TGAAGCTTTCAGAAATGAATGTG
CMTm30	$(AAG)_5$ + $(CAC)_7$	F: CAAACCATAACTTCCAG R: AGGTCCATATTTGACG
CMTp41	$(GCC)_8$ + $(CCT)_4$	F: GGAGGCCTTGGAATGATAGG R: TTCTCTCAACCACCGTCACC
CMTm61	$(GGA)_4$ + $(AAAA)_4$	F: GCCATTATTCCACTCCATGC R: TGCCTGCACCTGTTTTAGC
CMTp68	$(TC)_{10}$ + $(GGCTTC)_6$	F: ATTGATTGGGACGTGAGGAA R: CACACCCATTTCATTTTGACC
CMTm259	$(AG)_8$	F: ACCTCGAGGAAGCAAAAATG R: ATGGAGACGCGCAAGTAGAT

formulas given below. Allelic data were used to compute PIC value of SSRs, the codominant molecular marker system, using the Power Marker (Liu and Muse 2005) program (Anderson *et al.,* 1993). Genetic variation within genotypes was determined by Nei's gene diversity index (Nei, 1972), Shannon information index (Lewontin 1972), and the Popgen program (Yeh *et al.,* 1999). NTSYS-pc version 2.11f (Rohlf 1992) was used for the clustering analysis of the data set obtained from the SSR markers. The clustering was performed with the SAHN subprogram using the unweighted pair group method with arithmetic Mean (UPGMA) method. The STRUCTURE 2.2 program was used to determine the genetic structures of the genotypes (Pritchard *et al.,* 2000). In many genetic diversity studies with squash, genotypes are successfully separated into groups using the STRUCTURE program (Blair *et al.,* 2012; Hegay *et al.,* 2012). The F-statistic (FST) value reflects the variation between sub-populations (Zargar *et al.,* 2016). By using the GenAlex 6.5 software, principal coordinate analysis (PCoA) was performed to better understand the diversity among genotypes.

RESULTS AND DISCUSSION

Morphological properties of squash genotypes

In this study, 14 squash genotypes belonging to *Cucurbita maxima* Duch. were collected from different locations in Erzincan province. This squash population has been characterized according to morphological and molecular traits. Since changes in morphological traits occurred in response to external conditions, it is important to support these morphological variations with molecular studies. Morphological features of genotypes are given in Tables 3, 4 and 5. It was observed that the collected *Cucurbita maxima* Duch genotypes had different morphological characteristics in plant phenotype, leaf, flower and fruit characteristics. The plant growth habit was considered as trailing in 11 genotypes and semi-trailing in 3 genotypes. Branching was determined in all genotypes. The degree of branching was weak in 3 genotypes, moderate in 4 genotypes and strong in 7 genotypes. In addition, squash genotypes showed variation in terms of leaf characteristics such as leaf attitude of petiole, leaf blade size, incisions of leaf blade and intensity of leaf blade. Leaf attitude of petiole was identified as vertical in 11 genotypes and semi-vertical in 3 genotypes. Leaf blade size was large in 11 genotypes and medium in 3 genotype. Incisions was considered as weak in 3 genotypes and medium in 3 genotypes, whereas in 8 genotypes incisions of leaf blade were absent (Table 3). In many studies of Cucurbitaceae family, it has been emphasized that diversity is high in terms of morphologic characteristics (Balkaya *et al.,* 2010; Zhang *et al*., 2012; Wimalasiri *et al.,* 2016; Pratami *et al.,* 2019). As with other morphological features, it was observed that there was variation among genotypes in terms of flowers (male and female). It was determined that approximately 4 of the genotypes had ring at inner side of petal and that there was no inner circle in the female flowers of 10 genotypes. Based on the expression of inner circle color grade at petal of male flowers, genotypes are divided into 3 groups as absent, slight and medium. It was observed that 50% of genotypes (7 genotypes) did not have circle at inner side of petal. Genotypes were divided into 2 groups as yellow and orange according to color of pistil of male flower. It was determined that 8 genotypes had yellow and 6 genotypes had orange color. Hairiness on the flower stalk was considered as weak in 9 genotypes, medium in 3 genotypes and strong in 2 genotypes. Differences were determined between genotypes according to the length of the flower stalk. Genotypes were divided into 3 groups based on these properties. Only 1 genotype were classified as short, 10 genotypes as medium and 3 genotypes as strong (Table 4). In addition, squash genotypes showed high variation in fruit shapes and skin colours. It was determined that fruit shape of 9 genotypes were transverse wide elliptical, 3 genotypes were wide elliptical, 1 genotype were elliptical and 1 genotype was napiform. In a similar study of the same species; researchers have found that the fruit shapes of genotypes are globular, transverse elliptical, club and elliptical (Pevicharova and Velkov, 2017). Six different colors were determined as the major colour of skins of the squash genotypes: whitish (8 genotypes), grey (1 genotypes), grey-green (2 genotypes), grey-green-orange (1 genotypes), orange-green (1 genotypes) and cream-green (1 genotypes) (Table 5). These results confirm that fruit characteristics show a great deal of variation in the genus *Cucurbita* (Aruah *et al.,* 2010). In a similar study by Ferriol *et al.,* (2004) and Kaźmińska *et al.,* (2017) it has been determined that squash genotypes showed high diversity in terms of fruit characteristics. In breeding terms, species and genotypes of cucurbits have valuable morphological characters. Their diversity provides a good basis for a successful breeding program.

Table 3 Plant and leaf morphological parameters of squash genotypes

Genotypes	Plant			Leaf			
	Growth habit	Branching	Degree of branching	Leaf attitude of petiole	Leaf blade size	Incisions	Intensity of blade green color
≠5	Trailing	Present	Strong	Vertical	Large	Absent	Medium
≠12	Trailing	Present	Strong	Vertical	Medium	Absent	Medium
≠15	Semi trailing	Present	Weak	Semi vertical	Large	Weak	Dark
≠16	Trailing	Present	Weak	Semi vertical	Large	Weak	Dark
≠18	Trailing	Present	Strong	Vertical	Large	Absent	Dark
≠20	Trailing	Present	Medium	Vertical	Large	Medium	Dark
≠21	Trailing	Present	Medium	Vertical	Large	Weak	Dark
≠22	Trailing	Present	Medium	Vertical	Large	Medium	Dark
≠24	Trailing	Present	Strong	Semi vertical	Large	Absent	Dark
≠43	Trailing	Present	Strong	Vertical	Medium	Absent	Medium
≠44	Trailing	Present	Strong	Vertical	Large	Absent	Medium
≠45	Trailing	Present	Strong	Vertical	Large	Absent	Medium
≠47	Semi trailing	Present	Weak	Vertical	Medium	Medium	Medium
≠48	Semi trailing	Present	Medium	Vertical	Medium	Very strong	Medium

Table 4. Flower morphological parameters of squash genotypes

Genotypes	Female flower		Male flower			
	Inner side of petal	Pistil color	Petal inner circle color grade	Sepal leaf length	The length of the flower stalk	Hairiness on the flower stalk
≠5	Absent	Yellow	Slight	Medium	Medium	Weak
≠12	Absent	Yellow	Slight	Medium	Medium	Strong
≠15	Absent	Orange	Slight	Medium	Long	Weak
≠16	Absent	Orange	Absent	Medium	Medium	Medium
≠18	Absent	Yellow	Absent	Medium	Medium	Weak
≠20	Absent	Yellow	Slight	Medium	Medium	Medium
≠21	Present	Yellow	Absent	Medium	Long	Weak
≠22	Present	Orange	Absent	Medium	Long	Medium
≠24	Absent	Orange	Slight	Medium	Medium	Strong
≠43	Present	Yellow	Absent	Medium	Medium	Weak
≠44	Absent	Yellow	Absent	Medium	Medium	Weak
≠45	Absent	Yellow	Medium	Medium	Medium	Weak
≠47	Absent	Orange	Very strong	Medium	Short	Weak
≠48	Present	Orange	Absent	Medium	Medium	Weak

Table 5 Fruit morphological parameters of squash genotypes

Genotypes	Fruit					
	Shape	Main color of skin	Intensity of skin main color	Number of skin color	Diameter	Length
≠5	Transverse wide elliptical	Whitish	Light	One	Large	Long
≠12	Transverse wide elliptical	Whitish	Light	One	Large	Long
≠15	Elliptical	Grey	Light	One	Large	Long
≠16	Transverse wide elliptical	Grey green	Dark	One	Large	Medium
≠18	Transverse wide elliptical	Whitish	Light	One	Large	Long
≠20	Wide elliptical	Whitish	Light	One	Large	Long
≠21	Transverse wide elliptical	Whitish	Light	One	Large	Long
≠22	Transverse wide elliptical	Whitish	Light	One	Large	Medium
≠24	Wide elliptical	Whitish	Light	One	Large	Medium
≠43	Wide elliptical	Grey-green-orange	Light	Two	Large	Medium
≠44	Transverse wide elliptical	Grey green	Light	One	Large	Long
≠45	Transverse wide elliptical	Whitish	Light	One	Large	Long
≠47	Transverse wide elliptical	Orange-green	Medium	One	Medium	Short
≠48	Napiform	Cream-green	Light	Two	Small	Short

Table 6 Allele number, polymorphic allele number, polymorphism percentage and PIC values of iBPS markers.

Number	Primer	Number of Alleles	Major Allele Frequency	Gene Diversity	PIC*	He**
1	GMT-P41	3,000	0,857	0,314	0,162	0.143
2	GMT-M61	3,000	0,893	0,064	0,140	0.107
3	GMT-P68	5,000	0,833	0,212	0,190	0.214
4	GMT-M259	3,000	0,929	0,119	0,107	0.071
5	GMT-P18	3,000	0,929	0,183	0,107	0.036
6	GMT-P25	4,000	0,857	0,283	0,202	0.036
7	GMT-M30	2,000	1,000	0,093	0,000	0,000
Mean		**3,286**	**0,900**	**0.181**	**0.130**	**0.087**
Total		**23**				

***PIC-** Polymorphic information content, ****He** (expected heterozygosity)

SSR analysis

The SSR method has been successfully applied to various species to identify genetic relationships (Yu *et al.,* 2000; Shirasawa *et al.,* 2010; Mimura *et al.,* 2012; Kaźmińska *et al.,* 2017; Xu *et al.,* 2017; Miyatake *et al.,* 2019; Duman *et al.,* 2020). These markers have proven to effectively improve genetic diversity analysis and are very effective tools in genetic diversity and association studies due to their high polymorphic nature and transferability. (Ishii *et al.,* 2001; Ruizhen *et al.,* 2004; Jasim Aljumaili *et al.,* 2018). The 7 SSR markers used in our study produced a total of 23 polymorphic bands, the number of alleles per marker ranged from 2 (GMT-M30 marker) to 5 (GMT-P68 marker) and the mean number of alleles was 3.286 (Table 6). In similar studies of *Cucurbita* species, researchers have found the mean number of alleles amplified per SSR marker primers as 3 (Gong *et al.,* 2012; Duman *et al.,* 2020). In other studies of *Cucurbita maxima*, the number of alleles per marker ranged from 3 (CMTp208, CMTmC43, CMTp19, CMTp20, CMTp223, CMTm13 markers) to 10 (CMTp201) (Kaźmińska *et al.,* 2017). While some of the results are similar, some of them differ and to the results in our study. The differences are thought to be due to the markers and genotypes used. In many studies using SSR markers, it has been stated that SSR markers are successful to detect polymorphism and diversity in species belonging to the genus *Cucurbita* (Katzir *et al.,* 2000, Paris *et al.,* 2003; Gong *et al.,* 2012). The SSR markers were first of all explored and used for *Cucurbita moschata* and *Cucurbita pepo* (Gong *et al.,* 2008) and their transmissibility to other *Cucurbita* or other highly genetically distant *Cucurbita* species was concluded (Gong *et al.,* 2013; Kong *et al.,* 2014; Yildiz *et al.,* 2015). Polymorphic information content (PIC) is an important value that evaluates the efficiency of polymorphic loci and determines the discrimination ability of markers. The PIC value ranges from 0.00 (GMT-M30) to 0.202 (GMT-P68), with a mean of 0.13. The markers GMT-P25 and GMT-P68 were found to be the best among the markers used to discriminate between genotypes due to their higher PIC values (Table 6). In some studies, the PIC value changed according to the number of SSR markers used and the number of genotype and analysis method. In the study conducted by Kaźmińska *et al.,* (2017) the highest PIC value was reported as 0.51 in *Cucurbita maxima*. In other studies, with SSR markers, the PIC value was found between 0.49 and 0.75 for melon and between 0.18 and 0.64 for cucumber. Of the markers, PKCT111 was considered the most informative as it showed the greatest genetic variation (Katzir *et al.,* 1998). In a study conducted in Kenya with 96 pumpkin samples using SSR markers, the mean PIC value was determined as 0.49, and cluster analysis showed that the level of similarity between genotypes was high. (Kiramana *et al.,* 2017). Gene diversity ranges from 0.064 (GMT-M61) to 0.314 (GMT-P41), with a mean of 0.181. Genetic diversity among populations tends to be high in most self-pollination species and low in out pollination and shows a falling tendency species such as *Cucurbita* spp. (Kitavi *et al.,* 2014)

Cluster analysis and principal component analysis for SSR markers

Comparative analysis of molecular sequence data enables the determination of proximity or distance between genotypes as well as the construction of a phylogenetic tree for clustering genotypes. For this purpose, cluster analysis was performed between squash genotypes using UPGMA based on Nei's genetic distance. According to the results of this analysis, three major clusters were formed. Dice genetic similarity coefficient was used to estimate genetic diversity. This coefficient is often used to estimate genetic distance. The highest genetic difference was found between genotypes ≠5 and ≠48 genotypes. As a result of the analysis, squash genotypes were divided into three major groups. In the second group, only single genotype of Çayırlı location (≠18, ≠20) was determined. In the first cluster, two genotype was found Cevizli (≠47, ≠48) locations. In the third group, mostly genotypes of Bahçeliköy (≠5), Cevizli (≠43, ≠44, ≠45), Çayırlı (≠15, ≠16≠, ≠21, ≠22, ≠24) and Çatalarmut (≠12) locations were included. (Figure 2).

Principal coordinate analysis (PCoA) presents spatial distribution of relative genetic distance between the populations (Klaedtke *et al.,* 2017). In present study,

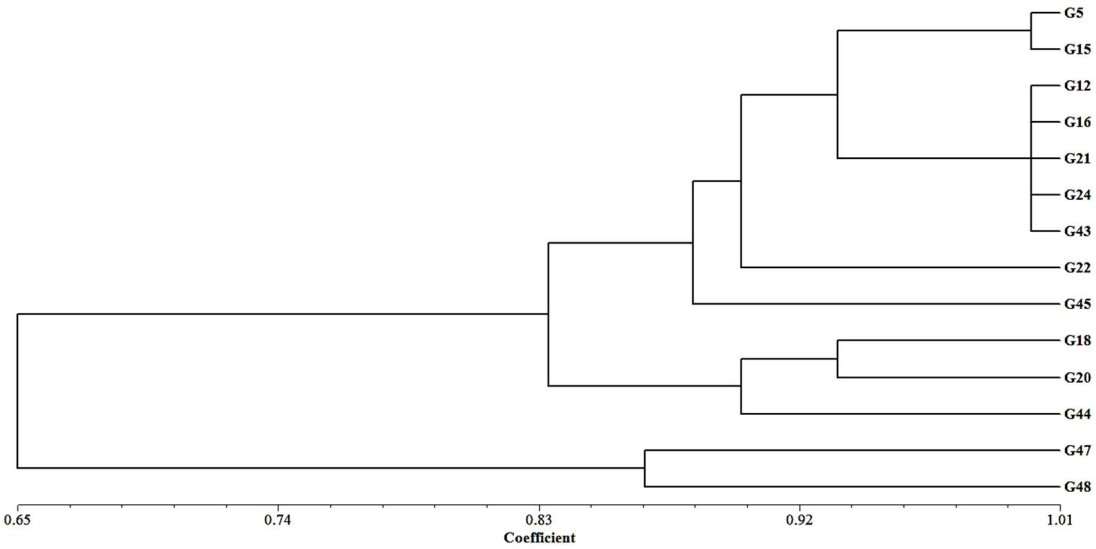

Fig. 2. Dendrogram generated by UPGMA method using SSR marker

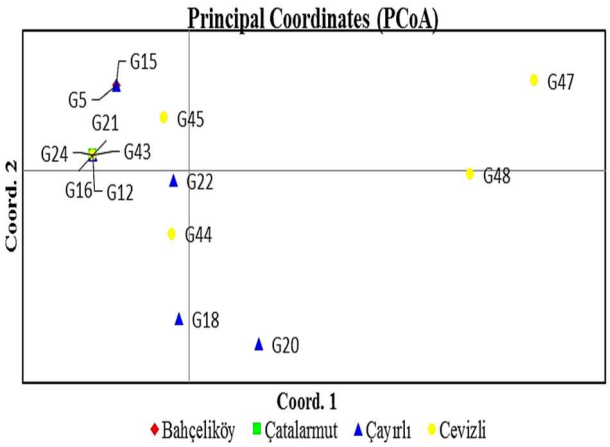

Fig. 3. PCoA created using the SSR marker and separated on 2-dimensional diagram

PCoA analysis was performed for better and more detailed visualization of the variation within and between the populations. With the aid this method, a 2-D diagram is generated based on closeness or distance matrix between the genotypes and the distances between the resultant groups put forth the actual distances (Mohammadi and Prasanna, 2003). According to present findings, the genotypes Çayırlı (≠22) and Cevizli (≠47, ≠48) were placed on upper left section of the Principle Axis-1. The genotypes Çayırlı (≠18, ≠20) and Cevizli (≠44) were gathered on lower left section of Axis-1. The genotypes Çatalarmut (≠6), Çayırlı (≠16, ≠21, ≠24) and Cevizli (≠43, ≠45) were placed on lower right section of Axis -1. The genotypes Bahçeliköy (≠5) and Çayırlı (≠15) were gathered on upper right section of Axis-1 (Figure 3).

Genetic structure analysis of SSR markers

ΔK is used to determine optimal values of K. The highest value in our study was obtained as K=3 (Figure 4). Several individuals revealed stronger genetic admixture of three genetic components (genotype ≠45, ≠22, and ≠44), and hence indicated the existence of gene flow. Similar results have been reported for the population structure of *Cucurbita maxima* genotypes in other studies using SSR markers (Kaźmińska *et al.,* 2017). In our study, 2 genotypes were found in the first subpopulation, 2 genotype in the second subpopulation and 10 genotypes in the third subpopulation (Figure 5; Table 7). The FST (F-statistics) values in the first, second and third subpopulations were determined as 0.4581, 0.5789 and 0.6112, respectively (Table 8).

CONCLUSION

Examination of morphological characterization within genotypes showed a wide variation of genotypes in terms of morphological characteristics (plant, flower, fruit, leaf). It was observed that 7 SSR markers used in squash genotypes yielded a total of 23 bands and the number of alleles per locus was 2.14. The present study demonstrate that the seven microsatellite markers were informative based on the mean PIC value of 0.130. The SSR marker GMT-P25 was the most polymorphic with a PIC value of 0.202. SSR markers with high PIC value are, therefore, potential candidate markers that can be used for genetic variation studies for squash genotypes (Nyabera *et al.,* 2021). Based on genetic structure analysis and UPGMA analysis, 3 groups were identified. Expanding our knowledge about genetic variation of genotypes is crucial for crossbreeding studies used to obtain lines resistant to various stress conditions or more productive varieties. Therefore, the assessment of genetic variability in the gene source is the first step, called pre-breeding, to improve and develop superior varieties. The SSR markers used were effective in distinguish among similar winter

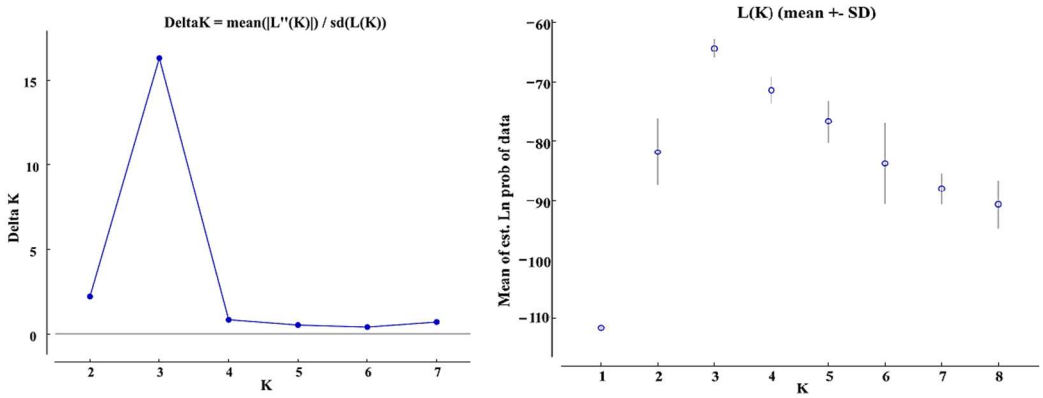

Fig. 4. Line plots from the mix model of Ln P(D) and ΔK structure for squash populations **a;** DK, **b;** The average value of the Ln P(D) statistic produced by the structure at each value of K.

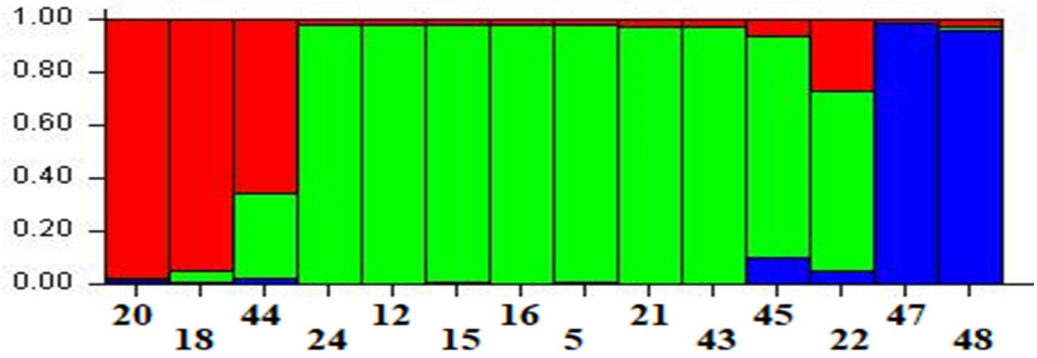

Fig. 5 Genetic structure of genotypes according to SSR data (*Cucurbita maxima* genotypes given in K = 3 are presented in Table 7)

Table 7 Membership coefficient of squash genotypes in four subpopulations.

	Sub-population		
Genotype	1	2	3
G5	0.012	0.376	0.611
G12	0.007	0.382	0.610
G15	0.014	0.375	0.611
G16	0.007	0.383	0.610
G18	0.008	0.595	0.397
G20	0.017	0.605	0.379
G21	0.007	0.383	0.611
G22	0.059	0.441	0.500
G24	0.007	0.382	0.611
G43	0.007	0.384	0.610
G44	0.025	0.527	0.447
G45	0.103	0.359	0.538
G47	0.987	0.007	0.006
G48	0.964	0.020	0.016

squash or pumpkin and therefore can be beneficial for consideration of *Cucurbita maxima* Duch. species diversity, screening of genetic resources and their selection. Considering the evaluated parameters, we can say that genotype 5 may be preferred for breeding programs. According to the results obtained, the genotypes with the greatest genetic distance in our study were 5 and 48. Genetically distant genotypes can be important for breeding because they can show heterosis. The results of this study suggest that SSR analysis can be used successfully in the estimation of genetic diversity among squash genotypes and potentially be included in future studies examining diversity in a larger collection of squash genotypes from various regions. It is thought that the results of this study will contribute to the existing squash cultivation and conservation of genetic resources in Turkey. The outcomes obtained in this study provide significant findings for the future in marker selection, characterization of genetic source, cultivation and selection of squash genetic source.

Table 8 Expected heterozygosity and FST values in four squash subpopulations

Sub-population (K)	Expected heterozygosity	F_{ST}
1	0,1610	0,4581
2	0,1331	0,5789
3	0,1167	0,6112
Mean	**0,1369**	**0,5494**

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