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Research Article

GENETIC ANALYSIS OF LOCAL PUMPKIN POPULATIONS

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ABSTRACT. Pumpkin is a tropical vegetable type in the *Cucurbitaceae* family. Selected types of summer squashes (*Cucurbita pepo*) are generally used in Turkey. This study was conducted to investigate the level of variation in different pumpkin genotypes, to determine genetic relationships and to determine linkage disequilibrium. In the study, 18 different inter-simple sequence repeats (ISSR) primers selected from a larger set of this primers were used for the genetic analysis of 140 different pumpkin genotypes obtained from Develi, Yeşilhisar and Tomarza districts of Kayseri province, where produces more than 30% of the Turkish pumpkin seed production. For 188 markers obtained with the 18 ISSR primers, the total polymorphism rate was determined as 98%. Based on the UPGMA analyses, it was determined that the similarity level of 140 genotypes had an average of 0.72 and the similarity index ranged between 0.47-0.97 and basically formed 2 main groups. The analysis of molecular variance (AMOVA) indicated that 68% of the total variation was within the population and 32% was between the populations. In linkage disequilibrium analyses, the number of markers associating with different markers was found to be variable. The results revealed that although most of the genetic variation resided within the population, a significant proportion of genetic variation presents among the population. This information may be valuable for pumpkin breeding programs.

Keywords: *Pumpkin, ISSR, AMOVA, NTSYS, ARLEQUIN*

INTRODUCTION

In recent years, seeds and nuts have received increasing attention due to the health effects of their bioactive components [1, 2, 3]. Nuts and seeds, including pumpkin seeds, are consumed regularly every day [4]. For these reasons, the use of pumpkin seeds as a snack is increasing. Pumpkins are a type of warm climate vegetable in the *Cucurbitaceae* family [5]. Pumpkin seeds are generally cultivated with *Cucurbita pepo*. In addition, *C. maxima*, *C. moschata*, *C. mixta* and *C. stilbo* species are used for the cultivation of pumpkin seeds for snacks [6, 7, 8]. In the cultivation of pumpkin as a snack, there is no need for irrigation, it can be cultivated in arid conditions, it can be suitable for crop rotation, it is easy to harvest, and the cultural processes can be done by machine to a large extent, increasing the production of pumpkin seeds.

Pumpkin seeds are very important for human nutrition. They are rich in oil, protein, mineral substances and amino acids [9]. They are a good source of magnesium, phosphorus, zinc, iron, calcium, sodium and copper [10, 11]. In addition, high levels of vitamin E and carotenoids [12], saponins [13], phenolic compounds [11], triterpenoids [8], phytosterols [7], unsaturated fatty acids and flavonoids [14, 15]. Approximately 75% of pumpkin seed oil is the unsaturated fatty acids linoleic, oleic, stearic and palmitic acid [1, 16]. It is also reported that squalene, a cholesterol-lowering hydrocarbon, is found in pumpkin seeds [6].

Food and Agriculture Organization (FAO) lists only pumpkin production for cooking instead of for snack. Turkey's total seed pumpkin production started in 2005 in Turkey and reached 55.043 tons in 2018. Reasons for sharp increase in pumpkin seed production in

Turkey are its relatively short vegetation period (120-160 days) and its suitability for rain-fed culture in Central Anatolia. In the production of pumpkin as a snack, Kayseri ranks first with a production of 16,751 tons, Nevşehir ranks second with a production of 16,403 tons, and Konya ranks 3rd with a production of 8,982 tons [17].

Pumpkins, which are produced in different regions of Turkey and mostly in arid conditions, are extremely diverse in terms of fruit size, shape, and color. DNA data on pumpkin genetic resources are insufficient. The first step in achieving breeding goals in plant materials is the conservation and recognition of genetic resources. The most important biomarkers used for this purpose are DNA markers. Genetic studies on horticultural crops have been successfully carried out with many DNA techniques developed [18, 19, 20, 21]. The ISSR technique, developed based on DNA polymorphism, is based on amplification of regions between reverse arranged, closely spaced microsatellites. ISSR markers, which are useful and easy to apply, are especially suitable for phylogenetic studies, evaluation of genetic diversity and identification of cultures [22].

In recent years, ISSR primers have been used successfully in studies conducted to determine the genetic relationship in different pumpkin species in the world [20, 23, 24, 25, 26]. In cucurbits, it is very important to carry out molecular characterization studies in addition to morphological characterization methods. Because farmers do not use hybrid or standard varieties when growing pumpkins, they mostly plant their own seeds next year. The level of variation within the same genotype source is unknown. This study was conducted to investigate the level of variation in different pumpkin genotypes, to determine genetic relationships and to determine linkage disequilibrium.

MATERIAL AND METHOD

Plant Materials

In this study, 140 pumpkin genotypes consisting of 14 different populations belonging to the *Cucurbitceae* family were used. Among the genotypes used in the study, 132 of them are different seed pumpkin genotypes obtained from Develi, Yeşilhisar and Tomarza districts of Kayseri province. The 8 genotypes are different pumpkin species used as outgroups (D26: *Lagenaria siceraria* (Develi/Kayseri); D28: *Cucurbita moschata* (Adıyaman); D34: *Cucurbita pepo* (Kayseri); D36: *Momordica charantia* (Aegean); D27: *Cucurbita pepo* var. *ovifera* (Yozgat); D29: *Cucurbita maxima* (Nevşehir); D35: *Citrullus lanatus* (Commercial); D37: *Citrullus lanatus* (Commercial). The seed samples of outgroups were kindly provided by Prof. Dr. Halit Yetişir of Erciyes University.

Molecular Methods

This study was carried out in the laboratories of Erciyes University, Faculty of Agriculture, Department of Horticulture. In the study, Doyle and Doyle's [27] CTAB total DNA extraction protocol modified by Gülşen et al. [28] was used. The 18 ISSR primers were used in molecular analysis. The total volume for the PCR reaction was prepared as 15 µl: 7.15 µl distilled water, 1.5 µl 10 x DNA polymerase buffer, 1.2 µl dNTPs (2.5 mM), 1 µl primer (5 mM), 0.15 µl Taq Polymerase (10 u/ml) and 20 ng of DNA.

Data were evaluated with NTSYS-pc (Numerical Taxonomy Multivariate Analysis System) version 2.11software [29]. First, similarity indexes between individuals were calculated with *DICE* analysis [30]. Then, a dendrogram was created using the UPGMA method using similarity indices. AMOVA [31] analyzes nested in ARLEQUIN software [32] were performed to examine genetic diversity at different hierarchical levels (between and

within populations). Linkage disequilibrium (LD) analyzes were performed to detect non-random linkages between the markers detected in pumpkin populations.

RESULTS AND DISCUSSION

PCR studies were performed on 140 cucurbits with 18 ISSR primers. Of the 188 markers obtained, 182 were identified as polymorphic and the total polymorphism rate was determined as 98%. While the highest number of markers was obtained from primer $(CA)_6AC$ with 17 pieces, the lowest number of markers was obtained from primer $(GT)_6GG$ with 3 markers (Table 1). In the study, the polymorphism rate in the pumpkin collection, which includes genotypes distributed in and around Kayseri, was found to be quite high (98%).

According to the UPGMA dendrogram obtained in the study, the similarity index between pumpkin genotypes varied between 0.47 and 0.97 (Fig 1). While the most distant genotypes were between D36 and 11, the most similar genotypes were 51 and 53, 104 and 105, 16 and 15, 212 and 213, 219 and 210. It was determined that the genotypes in the outgroup (D26, D27, D28, D29, D34, D35, D36, D37, 38) were located separately (Fig 1). The genotypes collected from the producers in the second main group were included in the same cluster, which is expected. It has been determined that some genotypes are located outside of the clusters, since pumpkins are completely open to foreign pollination due to being a monoic plant. Probably, those *C. pepo* genotypes are products of inter-species hybridization occurring naturally. The low similarity values detected among local genotypes indicate that their genetic diversity may be at a high level. These results are consistent with previous findings. Inan et al. [33] determined genetic relationships between *C. pepo* samples, the genetic similarity coefficients were found between 0.07 and 0.96 in ISSR analysis.

Similar studies in cucurbits with the ISSR molecular marker method are consistent with our study. The rate of polymorphism with ISSR markers in *Momordica charantia* L. was found to be 78.4% [34]. Rana et al. [35] on the other hand, found a polymorphism rate of 86.20% in the same species. Additionally, Karaman et al. [18] determined the polymorphism rate of 52% and similarity index of 0.80-0.98 in bitter melon (*Momordica charantia*).

On the other hand, the genetic diversity of *Cucurbita* genotypes from different origins was determined using other molecular markers such as RAPD [36, 37], SSR [38, 39, 40, 41] and the results were valuable for further germplasm characterization in different species in pumpkins. Other molecular marker techniques used have shown similar results to this study.

Table 1. *Primer Names Used in This Study, Total Number of Bands, Number of Polymorphic Bands and Polymorphism Rate (%)*

	Primer name	Number Of Scored Bands	Number Of Polymorphic Bands	Polymorphism Rate
1	$DBDA(CA)_7$	9	9	%100
2	$(CT)_8TG$	5	5	%100
3	$(GT)_8YA$	9	9	%100
4	$(CA)_8R$	10	10	%100
5	$VHVG(TG)_7$	10	10	%100
6	$(TCC)_5RY$	9	9	%100
7	$HVH(CA)_7T$	12	12	%100
8	$(AG)_7YC$	10	10	%100
9	$HVH(TCC)_7$	14	14	%100
10	$(CAC)_3G\ C$	15	15	%100
11	$(GT)_6GG$	3	3	%100
12	$(AGC)_6G$	11	10	%91
13	$BDB(CA)_7C$	14	12	%86
14	$(GACA)_4$	14	14	%100
15	$(AG)_8T$	11	11	%100
16	$(GA)_8YG$	8	8	%100
17	$(CA)_6AC$	17	16	%94
18	$(CAC)_6$	10	9	%90
	TOTAL	**191**	**186**	
	MEAN	**10,6**	**10,3**	**%98**

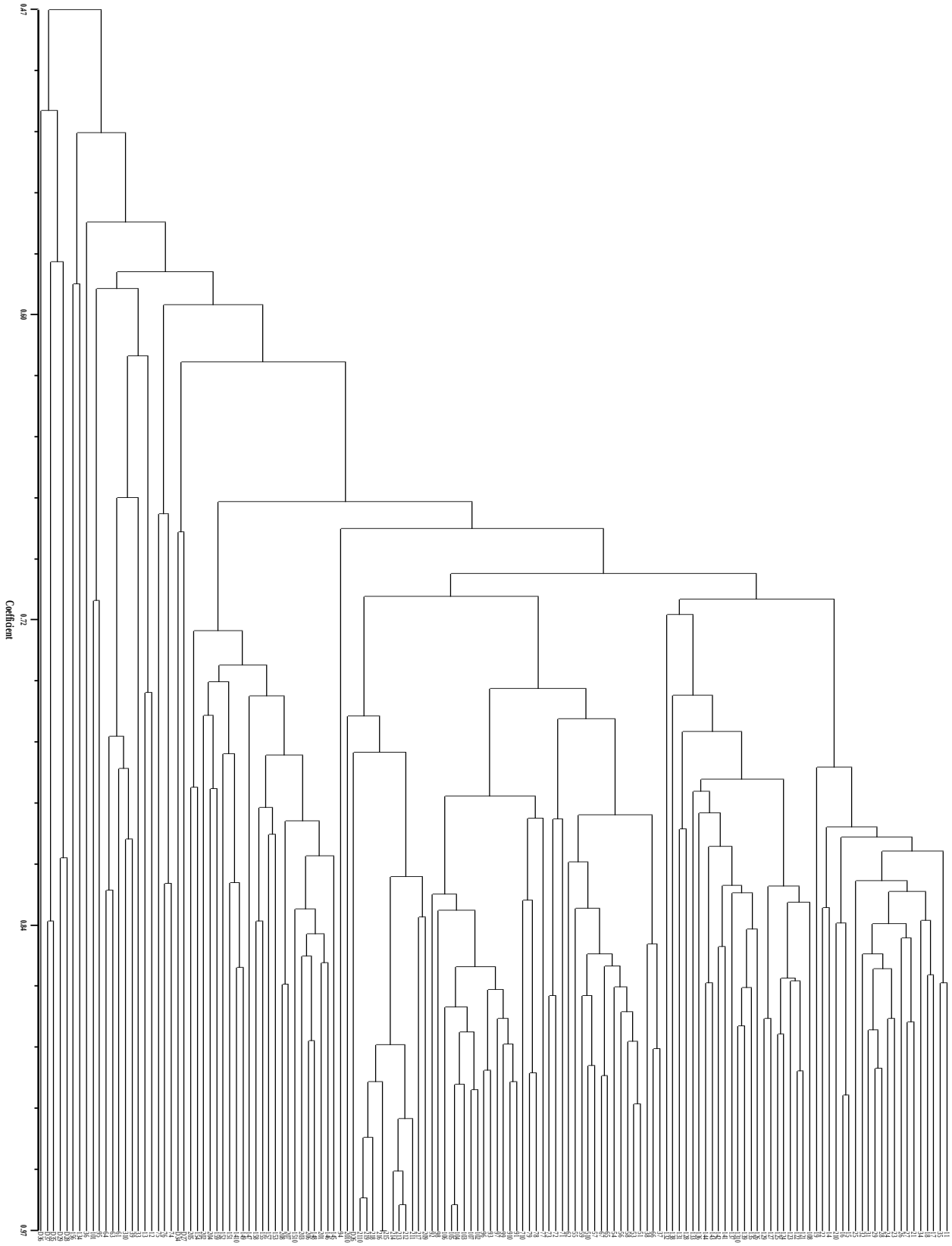

Fig 1. *UPGMA dendrogram created by using DICE similarity index in 140 pumpkin populations*

In addition, in this study, the AMOVA analyses were carried out using the ARLEQUIN 3.5 package program to examine genetic diversity at different hierarchical levels (between

populations and within populations). As a result of standard AMOVA, it was determined that intra-population variation explained 68.4% of the total variation, while inter-population variation explained 31.6%. This result shows that pumpkin breeding programs should be considered at both the inter- and intra-population levels. The mean difference between the populations calculated according to the mean distance method ranged from 0.10 to 0.68. The largest (0.68) difference was found between populations 9 and 14, while the smallest (0.10) difference was between populations 1 and 3 and populations 10 and 9. Population 14 was found to be quite different from other populations. The mean genetic difference average was calculated as 0.5. This population is the population containing the outgroups and is probably the most diverse population for this reason. This result also reveals that the effectiveness of this study is high.

As a result of linkage disequilibrium (LD) analysis, no correlation was found between loci 10 and 13 according to D, D', r2 and Chi-square P values. In this way, by examining other loci, it can be determined whether there is a connection between any two loci according to the *P* value. The number of linked loci per polymorphic locus and the significant linkage disequilibrium obtained for the populations were found to be low in the population of individuals belonging to the same subspecies, and weak linkage was present. A similar result was found by Öcal et al. [42] in watermelon plant. The number of loci associated with 77 polymorphic loci in ISSR analyzes (at 5% significance level), the total number of alleles per locus ranged from 2 to 8.

The linkage disequilibrium method can be defined as the non-random association of alleles at different loci in a population and is revealed by factors such as selection and genetic orientation. In the absence of mutation, migration, or selection, linkage will be in equilibrium at polymorphic loci, whereas linkage, crosstalk, and selection increase the level of LD [43]. Since pumpkin is a foreign pollinated species, low LD is expected.

Table 2. *Results of the AMOVA performed by using ARLEQUIN software*

Variation Source	Sum of Squares	Variation Components Variation (%)	
Between Populations	1423.6	9.5	31.6
Within Population	2303.5	20.4	68.4
Total	3727.1	29.9	100.0

Table 3. *The matrix showing the distances between 14 populations used in the study*

Total					3727.1				29.9				100.0		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0.00														
2	0.22	0.00													
3	0.10	0.23	0.00												
4	0.39	0.50	0.28	0.00											
5	0.21	0.38	0.36	0.20	0.00										
6	0.30	0.41	0.15	0.36	0.17	0.00									
7	0.32	0.48	0.25	0.41	0.28	0.17	0.00								
8	0.29	0.42	0.19	0.40	0.28	0.16	0.07	0.00							
9	0.37	0.47	0.24	0.51	0.35	0.32	0.43	0.32	0.00						
10	0.24	0.41	0.20	0.44	0.27	0.25	0.38	0.29	0.10	0.00					
11	0.25	0.37	0.15	0.33	0.16	0.17	0.30	0.21	0.20	0.16	0.0				
12	0.42	0.55	0.27	0.47	0.27	0.28	0.41	0.39	0.44	0.37	0.14	0.00			
13	0.35	0.47	0.20	0.40	0.24	0.22	0.37	0.32	0.39	0.32	0.08	0.16	0.00		
14	0.53	0.65	0.40	0.62	0.50	0.51	0.53	0.53	0.68	0.58	0.48	0.56	0.47	0.00	
15	0.36	0.48	0.20	0.44	0.25	0.26	0.36	0.33	0.43	0.34	0.28	0 .28	0.27	0.48	0.0

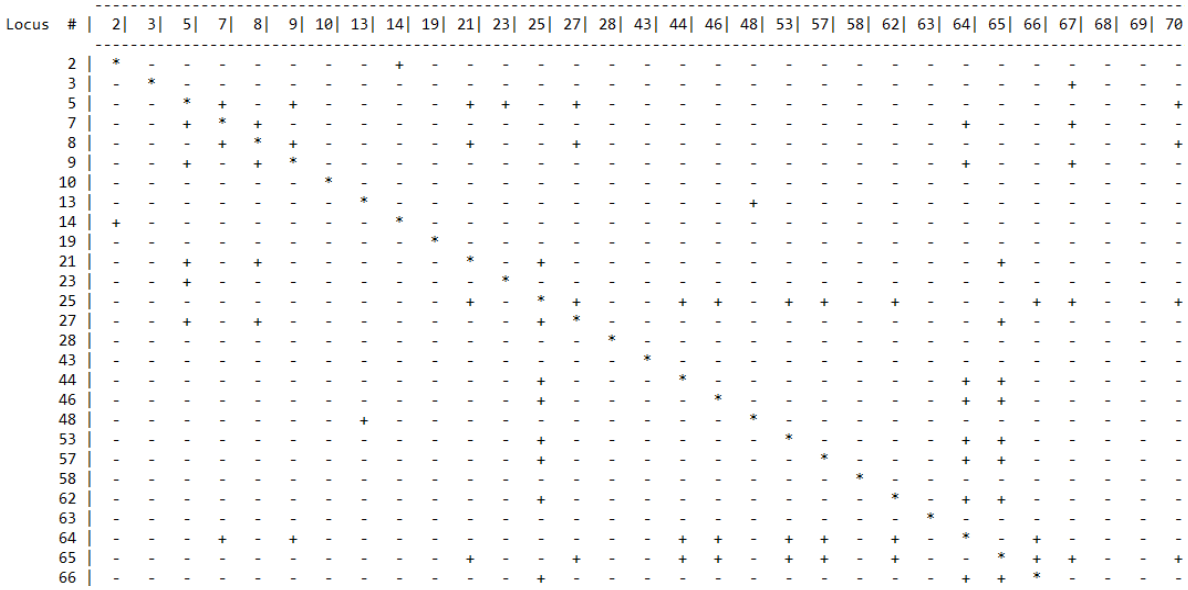

Fig 2. *Table of Significant Linkage Disequilibrium at significance level = 0.05. '+' and '-') indicates presence and absence of LD, respectively.*

Table 4. *Number of associated loci per polymorphic locus at significance level = 0.05)*

Locus	Adhesion Number	Locus	Adhesion Number	Locus	Adhesion Number	Locus	Adhesion Number
2	1	53	8	86	4	128	6
3	4	57	8	87	10	129	9
5	10	58	0	88	2	130	2
7	9	62	8	91	9	133	5
8	9	63	0	93	5	136	7
9	9	64	16	96	10	137	5
10	1	65	17	97	17	139	5
13	10	66	8	98	10	140	19
14	1	67	10	99	8	142	0
19	2	68	0	100	11	144	10
21	10	69	4	117	10	145	10
23	5	70	10	119	10	148	10
25	20	78	7	120	10	149	6
27	10	80	8	121	6	169	9
28	0	81	9	122	10	171	0
43	0	82	4	123	7	173	10
44	8	83	1	124	18	175	8
46	8	84	0	125	10	176	8
48	10	85	1	126	11	177	15
						178	16

CONCLUSION

In this study, genetic analysis of 140 different pumpkin genotypes was investigated by ISSR marker technique. pumpkin populations belonging *C. pepo*, which is widely grown in Kayseri region, was analyzed. It is aimed to reveal the relationships with the molecular characterization of the pumpkins belonging to the population collected from the region. As a result, it was determined that pumpkin genotypes had a high level of polymorphism genetically, within and between populations. The use of molecular data plays an active role in the identification of pumpkin genetic lines, the elimination of problems arising from seeds, in the planning of future pumpkin breeding studies, revealing true genetic relationships.

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