Erzincan Üniversitesi
Fen Bilimleri Enstitüsü Dergisi
2022, 15(3), 942-952
ISSN: 1307-9085, e-ISSN: 2149-4584
Araştırma Makalesi

Erzincan University
Journal of Science and Technology
2022, 15(3), 942-952
DOI: 10.18185/erzifbed.1113553
Research Article

Determination of Genetic Diversity in Some Pumpkin Genotypes Using SSR Marker Technique

Ömer Faruk COŞKUN[1*]

[1]Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Horticulture, Hatay, Turkey

Received:07/05/2022, **Revised:** 16/08/2022, **Accepted:** 21/09/2022, **Published:** 30/12/2022

Abstract

Pumpkin (*Cucurbita pepo* L.) is one of the important vegetables in the *Cucurbita* genus of the *Cucurbitaceae* family. DNA markers can be used in the selection studies carried out on vegetables. Microsatellite DNA sequences, which are a very good source of polymorphisms for eukaryotic genomes, are used in the investigation of genetic diversity, the creation of genetic maps and variety determination. In this study, molecular genetic characterization determined by using 16 SSR markers in 47 pumpkin genotypes. A similarity coefficient between 0.68-1.0 was determined between genotypes. It was determined that three genotypes clustered separately from the others. It was concluded that SSR (Simple Sequence Repeats) markers are a good choice for assessment of genetic diversity and differentiation between genotypes. As a result of this study genetic structures of the pumpkin genotypes, and important data were obtained that can shorten the duration of breeding studies.

Keywords: *Cucurbita pepo*, SSR, Molecular characterization

Bazı Kabak Genotiplerinde Genetik Çeşitliliğin SSR Markör Tekniği Kullanılarak Belirlenmesi

Öz

Kabak (*Cucurbita pepo* L.), *Cucurbitaceae* familyasının *Cucurbita* cinsi içerisinde yer alan önemli sebzelerden biridir. Sebzelerde gerçekleştirilen seleksiyon çalışmalarında DNA markörlerinden yararlanılabilmektedir. Ökaryotik genomlar için çok iyi bir polimorfizm kaynağı olan mikrosatellit DNA dizileri, genetik çeşitliliğin araştırılmasında, genetik haritaların oluşturulmasında ve çeşit tayininde kullanılmaktadır. Bu çalışmada 47 kabak genotipinde 16 SSR markörü kullanılarak genetik karakterizasyon belirlenmiştir. Genotipler arasında 0.68-1.0 arasında benzerlik katsayısı tespit edilmiştir. Üç genotipin diğerlerinden ayrı olarak kümelendiği belirlenmiştir. SSR markörlerinin (Basit Dizi Tekrarları) genetik çeşitlilik ve genotipler arası varyasyonun değerlendirilmesi için iyi bir seçim aracı olduğu sonucuna varılmıştır. Bu çalışma sonucunda bazı kabak genotiplerinin genetik yapıları belirlenerek ıslah çalışmalarının süresini kısaltabilecek önemli veriler elde edilmiştir.

Anahtar Kelimeler: *Cucurbita pepo*, SSR, Moleküler karakterizasyon

*Corresponding Author: omerfaruk.coskun@mku.edu.tr
Ömer Faruk Çoşkun, https://orcid.org/0000-0001-5398-5737

1. Introduction

There are about 30 species in humans that provide the majority of food sources [1]. On the other hand, thousands of species that are potential food sources are not used. This situation has revealed the necessity of determining the characteristics of other plant species and their consumption in different ways. The *Cucurbitaceae* family includes 118 genera and 825 species [2]. Cucurbita genus constitutes the plant group with the highest morphological variation in the plant kingdom [3]. In this genus, there are three species (*Cucurbita pepo* L., *Cucurbita maxima* Duchesne and *Cucurbita moschata* Duchesne) that are cultivated economically [4]. *C. pepo* is a type of vegetable that is highly cultivated and whose fruits, seeds and flowers can be consumed. In Turkey, both edible and snack pumpkin belonging to the *C. pepo* type are grown. In the world, 27.962.742 tons of pumpkin is produced on an area of 2.019.564 hectares. While China ranks first in world pumpkin production with 7.433.743 tons, this order is followed by India (5.113.692 tons), Ukraine (1.268.270), Russia (1.143.127) and America (1.050.713 tons). Due to its favorable conditions, Turkey ranks seventh in the world with 698.051 tons of pumpkin production value in terms of pumpkin cultivation [5]. The production of pupmkin for snacks in Turkey was realized as 64.861 ton in 2021 [6].

Unripe and ripe fruits of pumpkin are used in human nutrition because they contain important bioactive components (polysaccharides, proteins, peptides, vitamins and sterols) [7,8]. Consumption of these fruits provides relief from constipation, cleanses the blood and is good for digestion [9]. Pumpkin seeds are also evaluated in the food and pharmaceutical industry [10] and are used in many cultures of the world as a snack in raw or roasted form due to their high nutritional value. In addition, these seeds are used as flavor enhancer in meals or in minced meat formulations [11]. Pumpkin seeds are beneficial for health due to important nutrients (phenotic compounds, phytosterol, polyunsaturated fatty acid) contained in them [12]. Pumpkin seeds are rich in unsaturated fatty acids, oleic and linoleic acids, and contain squalene, which has a cholesterol-lowering effect [13]. At the same time, pumpkin seeds are also important in terms of the high amount of essential amino acids [14]. For these reasons, it can be considered that pumpkin seeds are beneficial for human health.

Pumpkin is one of the most polymorphic species in terms of fruit characters. Studies on the genetic characterization of pumpkin species and genotypes with high morphological differences are scarce. The most important biomarkers used for genetic characterization are DNA markers. Genetic studies in horticultural crops have been successfully carried out with many DNA marker techniques developed [15-20]. Different marker techniques such as SSR, RAPD, SRAP, ISSR and AFLP were used to determine genetic diversity among *Cucurbita* species [17,21-24]. One of the molecular markers, SSR (Simple Sequence Repeat), is called "microsatellite" and has been widely used in genetic characterization studies in recent years. SSR is an important marker technique that can be used in genetic analyzes due to its codominant, easy use, high polymorphism, and ubiquitous advantages in the eukaryotic genome [25]. The use of molecular data in the identification of pumpkin lines can play an active role in revealing true genetic relationships. In this study, it was aimed to determine the genetic diversity among different pumpkin genotypes by using the SSR marker technique.

2. Material and Methods

In the study, a total of 47 pumpkin genotypes selected from Kayseri and Nevsehir province (in Turkey) were used as plant material, considering their plant and fruit characteristics (Table 1). In the study, CTAB total DNA extraction protocol was applied and 30 mg of plant tissue was used for each genotype. After isolation, the DNA concentration was adjusted to 10 ng/l by measuring with a 1% agarose gel. For SSR analysis, Danin -Poleg, [26], Watchawongpoiboon and Chungwongse, [27] and Jarret et al., [28] developed 16 of the SSR primers were used. In this study, the best optimized primer pairs determined by Coskun et al., [29] were used. Polymerase chain reaction optimized 15 μL reactions contained 50 ng template DNA, 10 nmol dNTPs, 10 nmol SSR primers, 5 U Taq DNA polymerase, 1.5 mL of 10X polymerase chain reaction (PCR) buffer (50 mM KCl, 10 mM Tris-HCl, 2.5 mM MgCl2, pH 8.3). Typical amplification parameters were used and PCR products (5 mL) were resolved on 6.5% polyacrylamide gels at 50 W for 2.5 h.

For PCR, DNA samples were denaturated for 5 minutes at 95 ˚C, and then kept for 45 cycles at 95 ˚C for 1 minute, at 55 ˚C for 30 seconds, and at 72 ˚C for 1 minute. Finally, the marker was amplified by keeping the PCR mixture at 72 ˚C for 6 minutes. PCR products were visualized by running on acrylamide gel. For this purpose, M13 forward and reverse primers were added to synthetically prepared SSR primers. In this way, PCR products labeled at 700 or 800 nm wavelengths could be observed in the Li-Cor gel system.

Genotypes were scored as 1, 0 and 9 (for missing data). These data were analyzed using NTSYS (Numerical Taxonomy Multivariate Analysis System, NTSYS-pc version 2.1, Exeter Software, Setauket, N.Y., USA) package program [30]. Similarity indexes between individuals were determined. A dendrogram was created using the UPGMA method by using similarity indices. With the analyzes made, the variation and similarity levels between the pumpkin genotypes used in the study were determined and the characteristics of the genetic structure were revealed.

Table 1. The test materials of *Cucurbita pepo (C. pepo) (n=47).*

No	Origin	Pumpkin taxon	No	Origin	Pumpkin taxon	No	Origin	Pumpkin taxon
1	Kayseri-Tomarza	*C. pepo*	17	Kayseri-Develi	*C. pepo*	33	Kayseri-Develi	*C. pepo*
2	Kayseri-Tomarza	*C. pepo*	18	Kayseri-Develi	*C. pepo*	34	Kayseri-Develi	*C. pepo*
3	Kayseri-Tomarza	*C. pepo*	19	Kayseri-Develi	*C. pepo*	35	Kayseri-Develi	*C. pepo*
4	Kayseri-Tomarza	*C. pepo*	20	Kayseri-Develi	*C. pepo*	36	Kayseri-Develi	*C. pepo*
5	Kayseri-Tomarza	*C. pepo*	21	Kayseri-Develi	*C. pepo*	37	Kayseri-Yeşilhisar	*C. pepo*
6	Kayseri-Tomarza	*C. pepo*	22	Kayseri-Develi	*C. pepo*	38	Kayseri-Yeşilhisar	*C. pepo*
7	Kayseri-Tomarza	*C. pepo*	23	Kayseri-Develi	*C. pepo*	39	Kayseri-Yeşilhisar	*C. pepo*
8	Kayseri-Tomarza	*C. pepo*	24	Kayseri-Develi	*C. pepo*	40	Kayseri-Yeşilhisar	*C. pepo*
9	Kayseri-Tomarza	*C. pepo*	25	Kayseri-Develi	*C. pepo*	41	Nevşehir-Ürgüp	*C. pepo*
10	Kayseri-Tomarza	*C. pepo*	26	Kayseri-Develi	*C. pepo*	42	Nevşehir-Ürgüp	*C. pepo*
11	Kayseri-Develi	*C. pepo*	27	Kayseri-Develi	*C. pepo*	43	Nevşehir-Ürgüp	*C. pepo*
12	Kayseri-Develi	*C. pepo*	28	Kayseri-Develi	*C. pepo*	44	Nevşehir-Acıgöl	*C. pepo*

13	Kayseri-Develi	*C. pepo*	29	Kayseri-Develi	*C. pepo*	45	Nevşehir-Kozaklı	*C. pepo*
14	Kayseri-Develi	*C. pepo*	30	Kayseri-Develi	*C. pepo*	46	Nevşehir-Kozaklı	*C. pepo*
15	Kayseri-Develi	*C. pepo*	31	Kayseri-Develi	*C. pepo*	47	Nevşehir-Kozaklı	*C. pepo*
16	Kayseri-Develi	*C. pepo*	32	Kayseri-Develi	*C. pepo*			

3. Results and Discussion

Within the scope of this study, the genetic analysis of 47 different pumpkin genotypes was investigated using the SSR marker technique. In some studies, it was stated that SSR was successful in detecting polymorphism in species belonging to the genus *Cucurbita* [31,32]. Katzir et al., [33] also determined in their study that the SSR technique was much better in revealing the kinship relationships of *C. pepo*. They determined that SSR primers developed in different cucurbit species could yield successful results in *C. pepo* genotypes.

In this study, PCR studies were carried out on 47 pumpkin genotypes selected with a total of 16 SSR primers (Table 2). Twenty-nine of the 49 bants obtained were determined as polymorphic. The total number of alleles per primer ranged from 2 to 5 (mean 4.7). The number of polymorphic alleles is again between 2 and 7 (mean 3.1). In terms of the total number of alleles obtained, CSTCC813 loci produced the most alleles (5). Paris et al., [32] identified 2-5 alleles in 45 *C. pepo* genotypes. In the study conducted by Paris et al., [34], 16-30 scoreable bands and 15-23 polymorphic bands were obtained from 6 SSR primer pairs. Stift et al., [25] determined an average of 4.4 alleles per locus using 22 primer pairs on 48 genotypes. In a study, a total of 56 bands were obtained by using 10 SSR markers among *C. pepo* genotypes [35]. In another study, a total of 43 bands varying between 123 bp and 285 bp were obtained with nine primers [36]. Kayak et al., [37] determined the number of bands per primer to be 3.9 using SSR markers in the pumpkin genotypes in their study. Meru et al., [38] obtained an average of 3.92 alleles per locus in their study with SSR primer on 29 pumpkin genotypes. In a study on *C. pepo* genotypes, an average of 4.12 bands was obtained from SSR primers [39]. In the genetic diversity study among genotypes including 47 *C. pepo* and 1 *C. foetidissima*, 85 (mean 3.2) of 271 primers were polymorphic [40]. Findings from these studies are consistent with this study (mean number of bands 4.7). Aslan et al., [17] obtained 52 scoreable bands with 18 SSR primers in their study. In this study, more scoreable bands and more bands per primer were obtained. It is expected that the number of alleles at similar rates will be detected in different studies with similar sample groups. Small differences may be due to the use of natural populations in this study or to the large number of repetitions of different primers used.

The total polymorphism rate obtained from primer pairs was found to be 59% (Table 2). In the study, the rate of polymorphism was found 59% in the pumpkin collection, which includes genotypes with geographical origins in and around Kayseri. Paris et al., [34] tried to determine the genetic relationships of 45 *C. pepo* genotypes and it was determined that 280 (63%) of a total of 448 easily scored bands were polymorphic. Mujaju et al., [36] obtained an average of 67.86% polymorphism in watermelon genotypes using the SSR technique. Kayak et al., [37] determined the polymorphism rate as 87.7% and the mean dissimilarity value as 0.28 by using SSR markers in the pumpkin genotypes in their study. The results from the present study are

consistent with results from previous molecular studies. Martins et al., [41] used SSR markers to determine genetic diversity using 54 *C. pepo*, 32 *C. maxima* and 21 *C. moschata* populations, and it was stated that a 100% polymorphism was observed. The obtained polymorphism value is higher than this study findings. This may be due to the presence of genotypes of different species in the gene pool. The polymorphism values obtained from this study revealed that SSR markers can be used successfully in determining genetic relationships in pumpkin.

Table 2. Informations of primer sequence, total number of alleles (TNA), number of polymorphic alleles (PAN) and polymorphism rate (PR-%).

Primer	Primer Sequence	References	TNA	NPA	PR
CSTCC813	F:GTTGTGCTCCCCAATAGTTG R:CACCACTTCTTCCACCGAA	[26]	5	5	100
CSJCT14	F:TTCCACGTTACATTGGACGA R:AGAATTCATGGCCTGCAGAT	[27]	4	4	100
CSCCT571	F: CCTTTCTGCTGTTTCTTCTTC R: CCTTTCTGCTGTTTCTTCTTC	[26]	2	2	100
CSJCT 191	F:ACAATGGCAGGTCAATTAGC R:CCTTGGGTTGTATCGAAGAC	[27]	2	0	0
CSTA050	F: GAATTATGCAGATGGGTCTT R:CAAGAAGATCAAATGATAGC	[26]	4	2	50
CSJCT 216	F:CAGTAGGAGGAAGTGGGTTC R: CTTACTCCAACCAACCCAAC	[27]	2	0	0
CMCTT144	F:CAAAAGGTTTCGATTGGTGGG R:AAATGGTGGGGGTTGAATAGG	[26]	2	0	0
CMTC51	F: ATTGGGGTTTCTTTGAGGTGA R:CCATGTCTAAAAACTCATGTGG	[26]	4	2	50
CSJCT 71	F:AATTCCATGGACATCCAGCCGAG R:CAGTGAAAGGCACTAAAGCGGAG	[27]	2	2	100
CSJCT 252	F:GATGGTGGAGATGGAATTGGGAT R: TTAGAGCTGGAACTCTCCGCAAC	[27]	4	2	50
CI.1-06	F: CACCCTCCTCCAGTTGTCATTCG R: AAGGTCAGCAAAGCGGCATAGG	[28]	4	2	50
CI.1-120	F: CGCGCGTGAGGACCCTATA R: AGCAATTGATTGAGGCGGTTCT	[28]	2	0	0
CSJCT 656	F: TCCTACAACTCAAAGGGCCAAC R: GAAGTGGAGTGGAGTGGAGTGA	[27]	2	2	100
CI.2-23	F: GAGGCGGAGGAGTTGAGAG R: ACAAAACAACGAAACCCATAGC	[28]	2	0	0
CSJCT 662	F: ACGTCGTAAAACCATCGGAGTC R: GCTTCCAAGCGTCAAAGGTATC	[27]	4	2	50

CSJCT 664	F: AAGTGGGCTCGATTGGAAGA R: CCGTCGCCTTTCTCAAGTTC	[27]	4	4	100
Total			49	29	

According to the UPGMA dendrogram obtained using the scoring data, the similarity index between the pumpkin genotypes varied between 0.68 and 1.00. Barzegar et al., [42] used 14 SSR primer pairs to detect genetic diversity among 26 local cultivars of *C. pepo*. The percentage of polymorphic loci estimated using the genetic diversity index and the information index revealed moderate or high genetic diversity. Yunli et al., [39] determined the similarity coefficient in pumpkin genotypes between 0.73 and 1.0 in their study using SSR primers. The results from the present study are consistent with results from previous molecular studies. The similarity coefficient values (0.68-1.0) obtained in this study were found to be in a similar range (0.64-0.93) with the values determined by Aslan et al., [17]. Inan et al., [24] with the aim of determining genetic relationships between *C. pepo* samples, the genetic similarity coefficients were found between 0.07 and 0.96 in ISSR analysis. The wider variation may be due to the wider frequency of genotypes or the use of different marker techniques. Meru et al., [38] determined the genetic distance in pupmkin genotypes between 0.08 and 0.76 in their study. A wider variation was detected in this study. This may be due to the use of different pumpkin subspecies as genetic resources. In this study, genetic diversity among *C. pepo* populations was found to be narrower than in some studies [24,38]. This may be because these populations congregate from a narrow location. This is probably on a small scale to show high genetic diversity. It may also be due to material ingestion or gene flow from a single Cucurbita species.

Among some of the pumpkin genotypes (2 and 26; 3, 4, 6, 8, 16, 27 and 39; 10, 12, 13, 15, 17, 38, 18, 32 and 21; 19 and 20; 5, 11 , 22 and 28; 9, 36, 39, 37, 29 and 30; 24 and 25) no genetic variation was detected. In this case, it was determined that a total of 35 genotypes could be collected in 8 different groups among themselves, the other 12 genotypes were determined with different genetic structures, and as a result, it was determined that 47 different genotypes could show 20 different genetic structures. According to the UPGMA dendrogram, it was determined that the pumpkin genotypes formed 6 different clusters. No polymorphism was determined among genotypes 45, 46 and 47 in the first cluster, and these three genotypes were clustered separately from other genotypes. There is one genotype (43) in the second cluster, two genotypes (23 and 41) in the third cluster, three genotypes (2, 26 and 34) in the fifth cluster, one genotype (1) in the sixth cluster, and the remaining 37 genotypes in the fourth cluster has received (Fig 1). The similarity between the thirty-seven genotypes in the fourth cluster is high. In the dendrogram obtained by Kayak et al., [37] using SSR markers in the pumpkin genotypes, the majority of the genotypes were clustered together, similar to this study findings. The pumpkin plant has a monoic plant structure and is open to foreign pollination. This may have caused some genotypes to be located outside of the clusters.

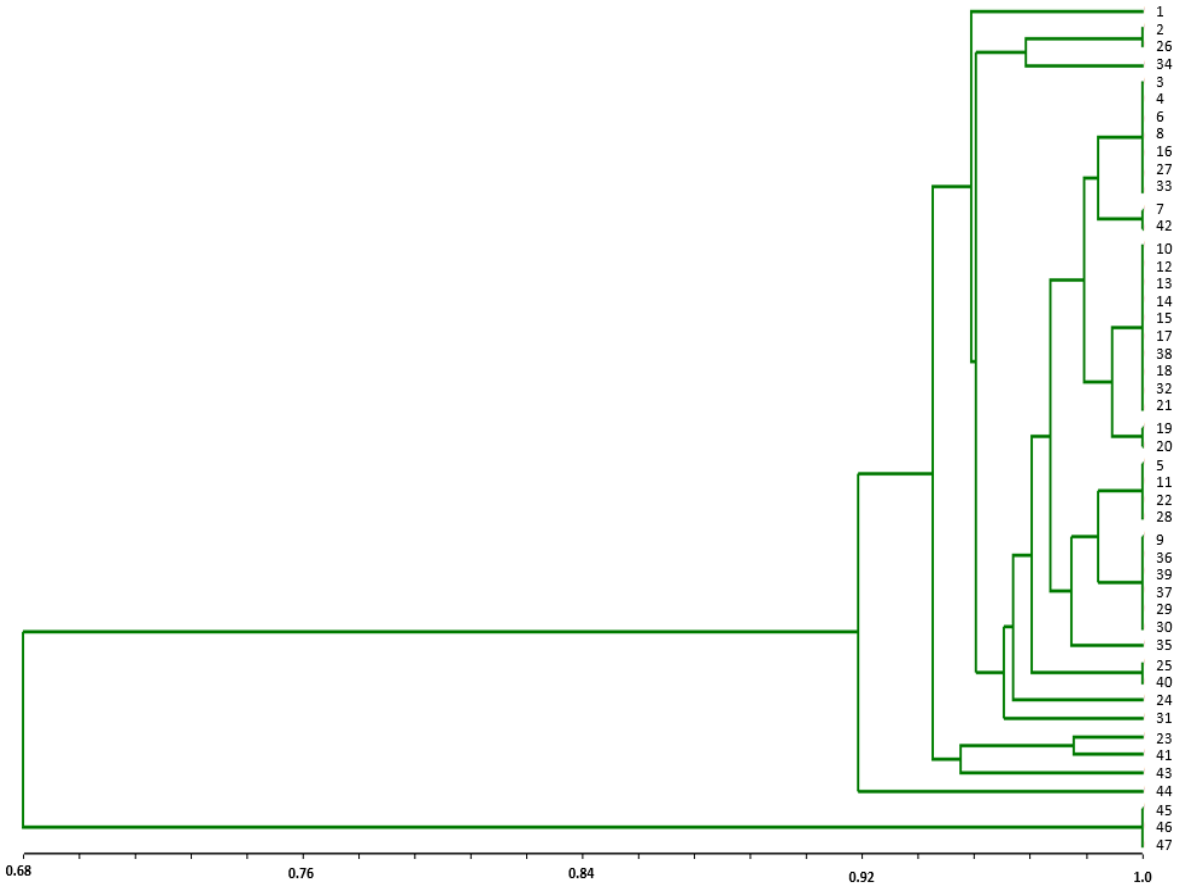

Figure 1. Dendrogram of resulting from a UPGMA cluster analysis based on SSR markers. The accessions correspond with the designations listed in Table 2.

Conclusion

Turkey is rich in genetic material, but the number of developed varieties is not high. Since market demands cannot be met, foreign varieties enter the country. This situation may cause a risky situation such as the extinction of native varieties. Although it is an important sector in Turkey, molecular studies have been carried out in a limited number of pumpkin. It is important to focus on variety development studies and to carry out genetic characterization studies for this purpose. It has been determined that SSR primers are suitable for pumpkin research. Successful primer pairs can be used in association mapping, purity testing and new marker development studies. Successful primers can be recommended for use in breeding programs. This study provided genetic information about *C. pepo* genotypes. These genotypes are an important source of diversity that can be used in breeding programs in the future. Determining the genetic diversity among Cucurbita species in Turkey, which is rich in plant diversity, may be useful in selecting and developing the most suitable genotype for use in genetic studies and breeding programs.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

References

[1] FAO, (2010) Second report on the state of the world's plant genetic resources for food and agriculture; UK Distributor, Stationery Office; FAO, Viale delle Terme di Caracalla: Rome, Italy, ISBN 925-106-5-349.

[2] Jeffrey, C., (1990) Systemetics of the Cucurbitaceae: An overview. In biology and utilization of the Cucurbitaceae; Bates, D. M., Robinson, R.W., Eds.; Cornel University Press: Ithaca, NY, USA, 3–9.

[3] Aruah, C. B., Uguru, M. I., Oyiga, B. C., (2010) Variations among some Nigerian Cucurbita landraces. African Journal of Plant Science, 4, 374–386.

[4] Maynard, D. N., Elmstrom, G. W., Talcott, S. T., Carle, R. B., (2002) El dorado'and'La estrella: Compact plant tropical pumpkin hybrids. American Society for Horticultural Science, 37, 831–833. doi: 10.21273/HORTSCI.37.5.831

[5] FAO, (2020) World pumpkin production list. http://www.fao.org/faostat/en/#data/QC. Access date: 13.02.2022

[6] TUIK, (2021) Bitkisel üretim istatistikleri. https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr, Access date: 13.02.2022

[7] Dar, A. H., Sofi, S. A., Rafiq, S., (2017) Pumpkin the Functional and therapeutic ingredient: A review, International Journal of Food Science and Nutrition, 168–173.

[8] Dotto, J. M., Chach, J. S., (2020) The potential of pumpkin seeds as a functional food ingredient: A review, Scientific African, 10, e00575. doi:10.1016/j.sciaf. 2020.e00575

[9] Bisognin, D. A., (2002) Origin and evolution of cultivated cucurbits. Ciencia Rural, 32, 715-723.

[10] Robinson, R. W., Decker-Walters, D. S., (1997) Cucurbits. In: Crop production department of horticultural science. Cornell Univ. and D.S. Decker-Walters, The Cucurbit Network U.S.A.

[11] El-Adawy, T. A., Taha, K. M., (2001) Characteristics and composition of watermelon pumpkin, paprika seed oils and flours. Journal of Agriculture and Food Chemistry, 49, 1253-1259. doi: 10.1021/jf001117+

[12] Fu, C. L., Shi, H., Li, Q. H., (2006) A review on pharmacological activities and utilization technologies of pumpkin. Plant Foods for Human Nutrition, 61, 73-80. doi: 10.1007/s11130-006-0016-6

[13] Ermis, S., (2010) The effect of ecology on seed production and snack quality of pumpkin (*Cucurbita pepo* L.) in Turkey. PhD, Ankara University, Graduate School of Natural and Applied Sciences, Ankara, Turkey.

[14] Glew, R. H., Glew, R. S., Chuang, L. T., Huang, Y. S., Millson, M., Constans, D., Vanderjagt, D. J., (2006) Amino acid, mineral and fatty acid content of pumpkin seeds (*Cucurbita* spp) and *Cyperus esculentus* nuts in the Republic of Niger. Plant Food for Human Nutrition 61, 49–54. doi: 10.1007/s11130-006-0010-z

[15] Karaman, K., Dalda-Şekerci, A., Yetişir, H., Gülşen, O., Coskun, O. F., (2018) Molecular, morphological and biochemical characterization of some Turkish bitter melon (*Momordica charantia* L.) genotypes. Industrial Crops and Products, 123, 93–99. doi: 10.1016/j.indcrop.2018.06.036

[16] Uzun, A., Cil, A., Yaman, M., Coskun, O. F., (2020) Genetic diversity and some fruit characteristics of quince genotypes collected from Kayseri region. Turkish Journal of Agriculture - Food Science and Technology, 8 (2), 318–323. doi: 10.24925/turjaf.v8i2.318-323.3012

[17] Aslan, N., Coskun, O. F., Dalda-Sekerci, A., Gulsen, O., (2021) Estimation of purity levels of pumpkin genotypes (*Cucurbita pepo* L.) using molecular marker. Mustafa Kemal University Journal of Agricultural Sciences, 26 (3), 759-769. doi: 10.37908/mkutbd.995779

[18] Morilipınar, E. O., Dalda-Sekerci, A., Coskun, O. F., Gulsen, O., (2021) Genetic analysis of local pumpkin populations. International Journal of Agricultural and Natural Sciences, 14 (3), 264-272.

[19] Kırac, H., Dalda-Sekerci, A., Coskun, O. F., Gulsen, O., (2022) Morphological and molecular characterization of garlic (*Allium sativum* L.) genotypes sampled from Turkey. Genetic Resources and Crop Evolution, 1-9. doi: 10.1007/s10722-022-01343-4

[20] Kamasak, S., Coskun, O. F., Sekerci, A. D., Gulsen, O., (2022) Microsatellite analysis in some watermelon (*Citrullus lanatus*) genotypes. International Journal of Agriculture, Environment and Food Sciences, 6 (1), 58-64. doi: 10.31015/jaefs.2022.1.10

[21] Ferriol, M., Pico, B., Nuez, F., (2003) Genetic diversity of a germplasm collection of *Cucurbita pepo* using SRAP and AFLP markers. Theoretical and Applied Genetics, 107, 271-282. doi: 10.1007/s00122-003-1242-z

[22] Gong, L., Stift, G., Kofler, R., Pachner, M., Lelley, T., (2008) Microsatellites for the genus Cucurbita and an SSR-based genetic linkage map of *Cucurbita pepo*. Theoretical and Applied Genetics, 117, 37-48. doi: 10.1007/s00122-008-0750-2

[23] Tsivelikas, A. L., Koutita, O., Anastasiadou, A., Skaracis, G. N., Traka-Mavrona, E., Koutsika-Sotiriou, M., (2009) Description and analysis of genetic diversity among squash accessions. Brazilian Archives of Biology and Technology, 52 (2), 32-38. doi: 10.1590/S1516-89132009000200003

[24] Inan, N., Yildiz, M., Sensoy, S., Kafkas, S., Abak, K., (2012) Efficacy of ISSR and SRAP techniques for molecular characterization of some Cucurbita genotypes including naked (hull-less) seed pumpkin. The Journal of Animal & Plant Sciences, 22 (1), 126-136.

[25] Stift, G., Zraidi, A., Lelley, T., (2004) Development and characterization of microsatellite markers (SSR) in Cucurbita species. Cucurbit Genetics Cooperative Report, 27, 61-65.

[26] Danin-Poleg, Y., Reis, N., Tzuri, G., Katzir, N., (2001) Development and characterization of microsatellite markers in Cucumis. Theoretical and Applied Genetics, 102, 61—72. doi: 10.1007/s001220051618

[27] Watcharawongpaibon, N., Chunwongse, J., (2008) Development and characterization of microsatellite markers from an enriched genomic library of cucumber (*Cucumis sativus*). Plant Breeding, 127, 74—81. doi: 10.1111/j.1439-0523.2007.01425.x

[28] Jarret, R. L., Merrick, L. C., Holms, T., Evans, J., Aradhya, M. K., (1997) Simple sequence repeats in watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai). Genome, 40(4), 433-441.

[29] Coskun, O. F., Gulsen, O., Dalda-Sekerci, A., Yetisir, H., Pınar, H., (2017) SSR marker analysis at some seed pumkin lines. Akademik Ziraat Dergisi, 6, 151-156.

[30] Rohlf, F. J., (2010) NTSYSpc: Numerical taxonomy and multivariate analysis system. Version 2.21j. Exeter Software, Setauket, New York.

[31] Gong, L., Paris, H. S., Nee, M. H., Stift, G., Pachner, M., Vollmann, J., Lelley, T., (2012) Genetic relationships and evolution in *Cucurbita pepo* (pumpkin, squash, gourd) as revealed by simple sequence repeat polymorphisms. Theoretical and Applied Genetics, 124, 875–891. doi: 10.1007/s00122-011-1752-z

[32] Paris, H. S., Yonah, N., Portnoy, V., Mozes-Daube, N., Tzuri, N., Katzir, N., (2003) Assesment of genetic relationships in *Cucurbita pepo* (Cucurbitaceae) using DNA markers. Theoretical and Applied Genetics, 106, 971-978. doi: 10.1007/s00122-002-1157-0

[33] Katzir, N., Tadmor, Y., Tzuri, G., Leshzashen, E., Mozes-Daube, N., Danin-Poleg, Y., Paris, H. S., (2000) Further ISSR and preliminary SSR analysis of relationships among accessions of *Cucurbita pepo*. In: Katzir, N., Paris, H.S. (Eds.), Proc Cucurbitaceae 2000, Acta. Hortic. 510. Israel, pp. 433–439.

[34] Paris, H. S., Portnoy, V., Mozes-Daube, N., Tzuri, G., Katzir, N., Yonash, N., (2004) AFLP, ISSR, and SSR polymorphisms are in accordance with botanical and cultivated plant taxonomies of the highly polymorphic *Cucurbita pepo*. Acta Horticulturae, 634, 167-173. doi: 10.17660/ActaHortic.2004.634.20

[35] Ntuli, N. R., Tongoona, P. B., Zobolo, A. M., (2015) Genetic diversity in *Cucurbita pepo* landraces revealed by RAPD and SSR markers. Scientia Horticulturae, 189, 192–200. doi: 10.1016/j.scienta.2015.03.020

[36] Mujaju, C., Sehic, J., Werlemark, G., Garkava-Gustavsson, L., Fatih, M., Nybom, H., (2010) Genetic diversity in watermelon (*Citrullus lanatus*) landraces from Zimbabwe revealed by RAPD and SSR markers. Hereditas 147, 142–153. doi: 10.1111/j.1601-5223.2010.02165.x

[37] Kayak, N., Türkmen, Ö., Uncu, A. T., Dal, Y., (2018) Characterization of edible seed pumpkin (*Cucurbita pepo* L.) lines by SSR (simple sequence repeat) markers. Manas Journal of Agriculture Veterinary and Life Sciences, 8 (2), 17-24.

[38] Meru, G., Leyva, D., Michael, V., Mainviel, R., Dorval, M., Fu, Y., (2019) Genetic variation among *Cucurbita pepo* accessions varying in seed nutrition and seed size. American Journal of Plant Sciences, 10, 9. doi: 10.4236/ajps.2019.109109

[39] Yunli, W., Yangyang, W., Wenlong, X., Chaojie, W., (2020) Genetic diversity of pumpkin based on morphological and SSR markers. Pakistan Journal of Botany, 52 (2), 477-487. doi: 10.30848/PJB2020-2(6).

[40] Wang, W., Shi, Y., Liu, Y., Xiang, C., Sun, T., Zhang, M., Shu, Q., Qiu, X., Bo, K., Duan, Y., Wang, C., (2021) Genetic relationships among *Cucurbita pepo* ornamental gourds based on EST-SSR markers. Czech Journal of Genetics and Plant Breeding, 57, 125−139. doi: 10.17221/27/2021-CJGPB

[41] Martins, S., Pinto, O., Carlos, C., Carvalho, de R., Carnide, V., (2015) Assessing genetic diversity in landraces of *Cucurbita* spp. using a morphological and molecular approache. Procedia Environmental Sciences, 29, 68-69. doi: 10.1016/j.proenv.2015.07.162

[42] Barzegar, R., Peyvast, G., Ahadi, A. M., Rabiei, B., Ebadi, A. A., Babagolzadeh, A., (2013) Biochemical systematic, population structure and genetic variability studies among Iranian Cucurbita (*Cucurbita pepo* L.) accessions, using genomic SSRs and implications for their breeding potential. Biochemical Systematics and Ecology, 50, 187-198. doi: 10.1016/J.BSE.2013.03.048