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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Molecular characterization of some parsley (*Petroselinum crispum* Mill.) genotypes

Bazı maydanoz (*Petroselinum crispum* Mill.) genotiplerinin moleküler karakterizasyonu

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ABSTRACT

Parsley (*Petroselinum crispum* Mill.), a vegetable whose leaves are consumed, has many benefits for human health. The first step of parsley breeding is the characterization of existing genotypes. In this study, genetic diversity of 12 different parsley genotypes was determined by ISSR (Inter Simple Sequence Repeat) marker system. In the study, 41 of the 130 bands obtained from 16 ISSR primers were found to be polymorphic. The similarity coefficients ranged from 0.86–0.99 for ISSR. The average polymorphism was 31.5% and the number of bands varied between 4 and 14. While the genotypes that are genetically closest to each other were determined as the 5th and 6th genotypes, the most distant genotypes were the 3rd and 8th genotypes. Findings from the present study showed that there were genetic variations among the parsley genotypes examined. The obtained data will enable more effective utilization of the parsley genotypes, the genetic differences of which have been determined for the future breeding programs.

ÖZET

Yaprakları tüketilen bir sebze olan maydanozun (*Petroselinum crispum* Mill.) insan sağlığına birçok faydası bulunmaktadır. Maydanoz ıslahının ilk adımı mevcut genotiplerin karakterizasyonudur. Bu çalışmada 12 farklı maydanoz genotipinin genetik çeşitliliği ISSR (Inter Simple Sequence Repeat) markır sistemi ile belirlenmiştir. 16 adet ISSR primerinden elde edilen 130 banttan 41'inin polimorfik olduğu tespit edilmiştir. ISSR için benzerlik katsayılarının 0.86–0.99 arasında değiştiği tespit edilmişitr. Ortalama polimorfizm %31.5 olup bant sayısı 4-14 arasında değişmektedir. Genetik olarak birbirine en yakın genotiplerin 5 ve 6 numaralı genotipler olduğu; en uzak genotiplerin ise 3 ve 8 numaralı genotipler olduğu belirlenmiştir. Çalışmada sonuçları, incelenen maydanoz genotipleri arasında genetik varyasyon olduğunu göstermiştir. Elde edilen veriler, gelecekteki ıslah programlarında genetik farklılıkları belirlenen maydanoz genotiplerinin daha etkin kullanılmasını sağlayacaktır.

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INTRODUCTION

Climate changes, increase in monoculture cultivation and unplanned agricultural practices cause a decrease in plant genetic resources and diversity. Conservation of plant genetic diversity of the cultivated species is very important for the sustainability of plant production (Tan & Inal, 2003). For this purpose, it is very important to determine the genetic resources and their characteristics in order to be evaluated.

Parsley (*Petroselinum crispum* Mill.) is a biennial herb belonging to the family Aliaceae (Umbelliferae) (Slighoua et al., 2021). *Petroselinum crispum* Mill is synonymous with *Petroselinum hortense* Hoffm, *Petroselinum sativum* Hoffm, *Apium petroselinum* L and *Apium crispum* Mill (Agyare et al., 2017). Known to date back to Ancient Rome, parsley is a type of vegetable grown on a global scale today. There are three main types of parsley. These are the flat-leaved (ssp. *neapolinatum*), curly-leaved (ssp. *crispum*) and turnip rooted type (ssp. *tuberosum*). Most of the local demand for parsley is met by the flat-leaf parsley type. It is seen as a strong income source that provides continuous income to parsley producers throughout the year. In economic terms, commercial parsley production is increasing day by day. Approximately 108 604 tons of parsley is grown in Turkey on a yearly basis (TUIK, 2021).

While parsley is used as an aromatic additive in vegetable and fruit salads, it is also used as a condiment in meals. Parsley is rich in essential oils and flavonoids. It is known that it has antioxidant (Farzaei et al., 2013; Abu-Serie et al., 2019), anti-inflammatory (Ezer & Arisan, 2006), antidiabetic (Farzaei et al., 2013) and diuretic (Campos et al., 2009) activities. It has been determined that parsley flavonoids can show anti-fatigue activity by regulating oxidative stress and intestinal microbiota (Wang et al., 2022). Thanks to its rich bioactive components, its rhizomes can be used as medicine (Staniszewska et al., 2021).

Determination of genetic diversity in plants is the most important part of plant genetics and product development programs. Molecular markers can be used in plant characterization, gene mapping, species identification, and selection studies with the support of markers and seed purity testing. PCR (Polymerase Chain Reaction)-based molecular marker techniques have become widespread due to the advantages of shortening the breeding period in genetic diversity and genetic structure analysis in vegetable species. Different DNA techniques are used for selection in breeding studies (Coskun et al., 2017; Karaman et al., 2018; Tecirli et al., 2018; Kırac et al., 2022). Inter-Simple Sequence Repeat (ISSR) is a molecular marker technique involving PCR amplifications of DNA. The ISSR technique is a fast, simple and inexpensive method that can be used to analyze the structure and genetic diversity of species and to determine genetic relationships between varieties (Ganopoulos et al., 2011). This technique has been successfully used in many genetic characterization studies in other vegetable species (Pinar et al., 2017; Aslan et al., 2021; Morilipınar et al., 2021) and including parsley (Abou El-Leel et al., 2017; Boutsika et al., 2021). In the literature review, it was determined that genetic structure studies in parsley cultivars and genotypes are very limited. In this study, it was aimed to determine the genetic diversity of different parsley genotypes to be evaluated in cultivar breeding studies.

MATERIALS and METHODS

Plant materials

The research was carried out in Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Horticulture. A total of 12 parsley genotypes were used in the study. The genotypes that are the subject of the study belong to the collection compiled from Hatay province and its districts, where the most of the parsley production is carried out in Turkey (Table 1).

Table 1. Genotype codes and geographic locations (province and district)
Çizelge 1. Genotip kodları ve coğrafik konumları (il ve ilçe)

No	Code	Location	No	Code	Location
1	HMKU-MA1	Hatay-Samandağ	**7**	HMKU-MA7	Hatay-Arsuz
2	HMKU-MA2	Hatay-Samandağ	**8**	HMKU-MA8	Hatay-Arsuz
3	HMKU-MA3	Hatay-Samandağ	**9**	HMKU-MA9	Hatay-Arsuz
4	HMKU-MA4	Hatay-Samandağ	**10**	HMKU-MA10	Hatay-Arsuz
5	HMKU-MA5	Hatay-Arsuz	**11**	HMKU-MA11	Hatay-Antakya
6	HMKU-MA6	Hatay-Arsuz	**12**	HMKU-MA12	Hatay-Antakya

Molecular study

DNA isolation was carried out by the method given in Doyle & Doyle (1990). The total volume for the PCR reaction was prepared as 15 µl: 9.0 µl distilled water, 1.5 µl 10 x DNA polymerase buffer, 1.2 µl dNTPs (2.5 mM), 1.1 µl primer (5 mM), 0.20 µl Taq Polymerase (1 U) and 2.0 µl of 20 ng DNA. The samples were analyzed using PCR mix and 16 ISSR primers. PCR cycling consisted of initial denaturation at 94 °C for 2 min, 35 cycles of 45 s denaturation at 94 °C, 1 min for annealing at the primer-specific melting temperature and 2 min of extension at 72 °C with a final extension at 72 °C for 7 min. PCR products were separated on 1.5% agarose gel at 110 V for 5 to 6 h and visualized under UV light. Polymorphic bands shown by ISSR marker primers were detected in the analyses. Genetic similarity values between the 12 parsley genotypes were determined using NTSYS (Numerical Taxonomy Multivariate Analysis System) package program. The genetic similarity coefficient was calculated according to Dice (1945) method and the dendrogram showing the similarities between the individuals was obtained by the UPGMA method. Principle Component Analysis (PCA) was also conducted with the use of this matrix in NTSYS software. Population structure was analyzed in STRUCTURE V2.3 program for K values ranging from 1 to 10 (Pritchard et al., 2000; Falush et al., 2003). Each running was repeated 5 times with 50 000 burn-in length. The most likely population ancestor was determined by Evanno's correction method (Evanno et al., 2005).

RESULTS and DISCUSSION

A total of 16 primers were used in the ISSR studies. The lowest number of bands (4) (GT6GG) and the highest number of bands (14) (GAA6) were obtained from the primers. The total number of bands obtained was 130 and the number of bands per primer was 8.12 (Table 2). No polymorphism was observed in primary GACA4. The highest rate of polymorphism (83.3%) was obtained from primer DBDACA7. 41 of the 130 bands obtained were polymorphic and the polymorphism rate was determined as 31.5% (Table 2). Band sizes varied between 115-1700 bp.

The similarity coefficients depending on the DICE index were determined with the NTSYS package program using ISSR primers in 12 parsley genotypes. The similarity coefficient range of genotypes varied between 0.86 and 0.99 (Table 3). The most distant genotypes were determined as the 3rd and 8th genotypes with a similarity coefficient of 0.86. The genetically closest genotypes were the 5th and 6th with a similarity coefficient of 0.99. At the same time, there was a high similarity coefficient between 10 and 11 genotypes (Table 3).

Table 2. Band profiles obtained using ISSR primers

Çizelge 2. ISSR primerleri kullanılarak elde edilen bant profilleri

Primer Number	Primer Name	Total Number of Bands	Number of Polymorphic Bands	Polymorphism Rate (%)
1	CT8TG	5	3	60.0
2	DBDACA7	6	5	83.3
3	BDBCA7C	9	3	33.3
4	HVHCA7T	5	1	20.0
5	AG7YC	7	1	14.3
6	GT8YA	9	1	11.1
7	AG8T	8	5	62.5
8	GACA4	8	0	0
9	VHVGTG7	10	2	20.0
10	CAC3GC	8	1	12.5
11	CAC6	12	6	50.0
12	AGC6G	5	4	80.0
13	CA6AC	11	1	9.1
14	GAA6	14	3	21.4
15	GT6GG	4	1	25.0
16	GA8YG	9	4	44.4
Total		**130**	**41**	
Mean		**8.12**	**2.56**	**31.5**

Table 3. Genetic similarity matrix based on DICE coefficient

Çizelge 3. DICE katsayısına dayalı genetik benzerlik matrisi

	1	2	3	4	5	6	7	8	9	10	11	12
1	**1.00**											
2	0.98	**1.00**										
3	0.94	0.95	**1.00**									
4	0.94	0.95	0.94	**1.00**								
5	0.98	0.98	0.95	0.96	**1.00**							
6	0.98	0.98	0.95	0.95	0.99	**1.00**						
7	0.98	0.97	0.94	0.94	0.98	0.98	**1.00**					
8	0.92	0.91	0.86	0.90	0.92	0.92	0.94	**1.00**				
9	0.94	0.94	0.91	0.95	0.96	0.95	0.95	0.94	**1.00**			
10	0.95	0.96	0.95	0.97	0.96	0.96	0.95	0.89	0.93	**1.00**		
11	0.95	0.96	0.95	0.96	0.95	0.96	0.95	0.88	0.93	0.99	**1.00**	
12	0.93	0.94	0.93	0.95	0.93	0.95	0.93	0.87	0.91	0.98	0.98	**1.00**

The similarity coefficients in the UPGMA dendrogram were determined as between 0.92 and 0.99 (Figure 1). In the cluster analyses, it was observed that the genotypes 8 and 9 were separated from the others. Among the other genotypes, three of them were clustered separately from the others. In the UPGMA dendrogram, four clusters were formed. Genotypes 1, 2, 5, 6, 7 were in the first cluster; genotypes 4, 10, 11, 12 were in the second cluster; genotype 3 was in the third cluster and the genotypes 8 and 9 were in the fourth cluster. The closest genotypes in the UPGMA dendrogram were determined as the 5th to 6th genotypes (Figure 1).

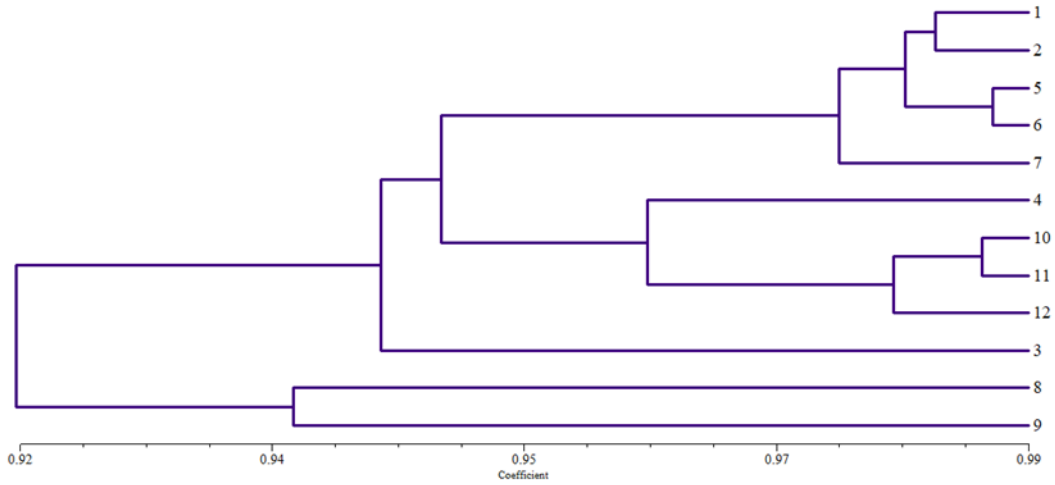

Figure 1. Dendrogram of the studied parsley genotypes based on the Dice similarity index
Şekil 1. İncelenen maydanoz genotiplerinin Dice benzerlik indeksine bağlı dendrogram

In the three-dimensional PCA graph, 5 (1, 2, 5, 6, 7) genotypes took place together and formed the first cluster (Figure 2). Genotypes 3, 4, 10, 11 and 12 formed the second closest cluster to these genotypes. Genotypes 8 and 9 separated from other genotypes and formed the other (third) cluster (Figure 2).

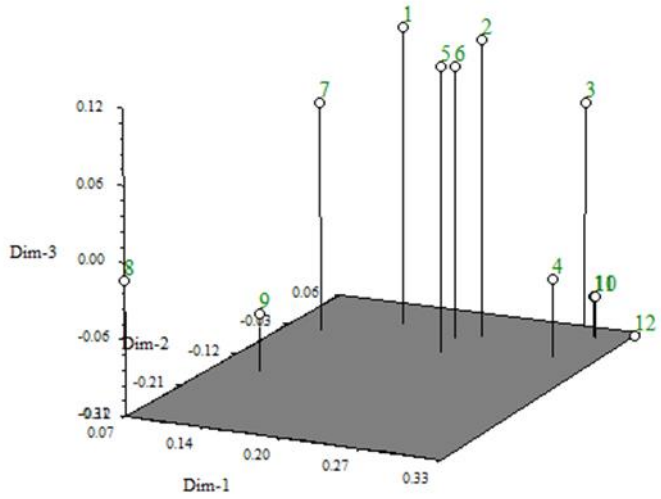

Figure 2. Three-dimensional graph obtained as a result of principal component analysis with ISSR
Şekil 2. ISSR ile temel bileşenler analizi sonucunda elde edilen üç boyutlu grafik

Considering the *K* values obtained with the ISSR data using the Structure Harvester program, it was determined that 12 parsley genotypes composed six subpopulations. The belonging rate of the four genotypes in the fifth sub-population and three genotypes in the sixth population was found to be 80% and above. The belonging coefficient of the one genotype (7) to the fifth subpopulation was 0.78 (Table 4). It can be said that the other four genotypes have mixed genetic structures (Figure 3).

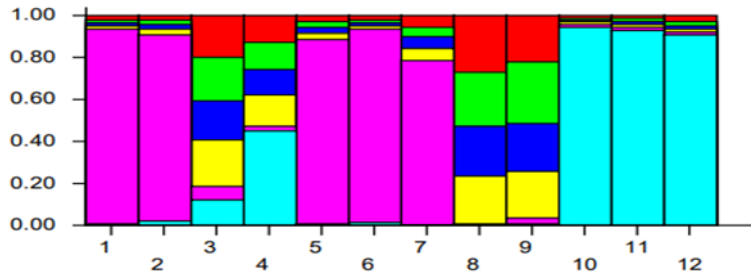

Figure 3. Graphical representation of the ISSR data and the membership coefficients obtained from the Structure program

Şekil 3. Structure programından elde edilen ISSR verilerinin ve üyelik katsayılarının grafiksel gösterimi

Table 4. Subpopulation membership coefficient values of the parsley genotypes

Çizelge 4. Maydanoz genotiplerinin alt popülasyon üyelik katsayı değerleri

Genotype	1st Sub-population	2nd Sub-population	3rd Sub-population	4th Sub-population	5th Sub-population	6th Sub-population
1	0.018	0.014	0.014	0.016	**0.930**	0.008
2	0.021	0.020	0.021	0.026	**0.888**	0.024
3	0.200	0.207	0.182	0.224	0.064	0.123
4	0.127	0.124	0.123	0.153	0.022	0.450
5	0.026	0.026	0.028	0.029	**0.880**	0.011
6	0.017	0.015	0.017	0.013	**0.921**	0.017
7	0.053	0.043	0.054	0.064	0.780	0.008
8	0.267	0.258	0.236	0.226	0.009	0.004
9	0.219	0.292	0.228	0.223	0.026	0.011
10	0.008	0.009	0.010	0.009	0.016	**0.947**
11	0.013	0.015	0.012	0.011	0.016	**0.934**
12	0.027	0.016	0.017	0.018	0.008	**0.914**

In some previous studies, genetic characterization studies were carried out on various parsley genotypes. In a study in Egypt, flat-leafed and curly-leafed two genotypes were examined (Ibrahim et al., 2017) while in a study in Iran, 15 different parsley genotypes were examined (Nasiri et al., 2015). In a study conducted in Greece, 24 parsley genotypes were examined (Boutsika et al., 2021) while genetic diversity analysis was performed on 32 parsley genotypes originating from different countries in a study conducted in Russia (Domblides et al., 2010). No genetic studies have been performed on parsley genotypes originating in Turkey. In this study, 12 parsley genotypes from the Hatay province of Turkey were analyzed.

Different marker techniques have been used in previous genetic characterization studies on parsley. In a study, the SRAP technique, which is a semi-codominant marker system was used (Nasiri et al., 2015) while another study employed ISSR and RAPD techniques (Ibrahim et al., 2017). In another study, ISSR and RAPD techniques (Domblides et al., 2010) were usedwhile ISSR and AFLP marker techniques (Boutsika et al., 2021) were used in another study. Researchers have revealed genetic variation by using different number of primers. ISSR has been the most studied marker technique in parsley. In our study, genetic characterization study was carried out using ISSR marker technique. Higher number of primers (16) was used than the number of primers used in other studies (Ibrahim et al., 2017; Boutsika et al., 2021).

The number of bands per primer obtained in this study was 8.12 and the number of polymorphic bands per primer was 2.56. Domblides et al. (2010) calculated the number of bands per ISSR primer as 11 and Boutsika et al. (2021)

calculated the number of bands per ISSR primer as 7.83. Similarly, in our study, the number of bands per primer was determined as 8.12. This shows that a high number of bands can be obtained with the ISSR technique. However, unlike the literature, the number of polymorphic bands per primer obtained from this study was lower than the values obtained in some previous studies. The number of polymorphic bands per primer was determined as 8.25 (Nasiri et al., 2015) and 9.8 (Ibrahim et al., 2017) and a higher number of polymorphic bands could be obtained from this study. The reason for the low mean number of polymorphic bands in this study may be related to the genetic structure of the examined genotypes. In addition, the increase in the number of primers used may have caused the average number of polymorphic bands to be lower.

In this study, the greatest distance was calculated as 0.08 according to the UPGMA dendrogram. Nasiri et al. (2015) similarly found the greatest distance between parsley genotypes to be 0.06. However, in the study of Domblides et al. (2010), these values were determined to be higher (0.292). In studies conducted with populations located in close locations, genetic distance was low and in studies conducted with populations located in distant locations, genetic distance was found to be high. It has been previously determined that the genetic diversity was higher in parsley genotypes of different geographical origins (Domblides et al., 2010; Nasiri et al., 2015; Ibrahim et al., 2017; Boutsika et al., 2021). In the present study, it can be said that there was genetic diversity among the 12 parsley genotypes in Turkey. The effectiveness of the ISSR primers in parsley was first determined by Domblides et al. (2010). In the current study, it was confirmed that the ISSR technique can be used in parsley genotypes.

High yield is one of the most important goals of parsley growers. Existing genetic resources are used in vegetable breeding studies. Molecular characterization studies show that parsley has genetic diversity. Conservation of parsley genetic resources and increasing genetic diversity will enable the improvement of current production levels. In this respect, it is important to protect Turkey's parsley genetic resources and to determine genetic structures. The data obtained as a result of this present study will contribute to future genetic and breeding studies and it will be possible to use them in marker assisted selection (MAS) studies.

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STATEMENT OF CONFLICT OF INTEREST

The author(s) declare no conflict of interest for this study.

AUTHOR'S CONTRIBUTIONS

The contribution of each author is equal.

STATEMENT OF ETHICS CONSENT

Ethical approval is not applicable because this article does not contain any studies with human or animal subjects.

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