Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 14(4), 1731-1741, 2024
Journal of the Institute of Science and Technology, 14(4), 1731-1741, 2024

ISSN: 2146-0574, eISSN: 2536-4618

Moleküler Biyoloji ve Genetik / *Moleculer Biology and Genetic* **DOI: 10.21597/jist.1544279**

Araştırma Makalesi / *Research Article*

Geliş tarihi / *Received*: 06.09.2024 **Kabul tarihi / *Accepted*: 02.10.2024**

Atıf İçin: Çevik, G. ve Eren, B. (2024). Iğdır İlinde Yetiştiriciliği Yapılan Bazı Mısır (*Zea mays* L.) Genotiplerinin Moleküler Karakterizasyonu. *Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 14(4), 1731-1741.

To Cite: Cevik, G. & Eren, B. (2024). Molecular Characterisation of Some Corn (*Zea mays* L.) Genotypes Growing in Iğdır Province. *Journal of the Institute of Science and Technology*, 14(4), 1731-1741.

Iğdır İlinde Yetiştiriciliği Yapılan Bazı Mısır (*Zea mays* L.) Genotiplerinin Moleküler Karakterizasyonu

Gizem CEVİK[1], Baris EREN[2*]

Öne Çıkanlar:
- Filogenetik
- İPBS

Anahtar Kelimeler:
- Moleküler markör
- Mısır
- Zea mays L.

ÖZET:

Retrotranspozonlara dayalı primerler arası bağlanma bölgesi veya iPBS retrotranspozon belirteçleri, çok sayıda organizmada genetik çeşitliliğin belirlenmesinde yararlı olmuştur. DNA'yı izole etmek için CTAB tekniği kullanılmış ve genetik çeşitlilik çalışmaları yapmak için iPBS moleküler belirteçleri kullanılmıştır. Analizlerin bulgularına göre, genotipler %100 polimorfik lokus yüzdesi ile önemli derecede genetik çeşitlilik sergilemiştir. 12 IPBS markörü ile yürütülen moleküler tanımlama deneyleri sonucunda toplam 154 polimorfik bant üretilmiştir. Tipik polimorfizm oranı %100 olarak belirlenmiştir. Ayrıca, çalışmada incelenen tüm markörlerin gen çeşitliliğini ölçen ortalama polimorfizm (PIC) değeri 0,228 ve ortalama H değeri 0,274 olarak belirlenmiştir. DICE benzerlik katsayıları karşılaştırıldığında, 1 ve 14 numaralı genotipler 0,1600 katsayı değeri ile en düşük benzerlik oranını sergilemiştir. Bu sonuçlar, örneklerin DICE benzerlik katsayılarının karşılaştırılmasıyla elde edilmiştir. Analiz, 4 ve 3 numaralı genotipler arasındaki en büyük benzerlik değerinin 0.6747 olduğunu belirledi. Mısır genotipleri dört farklı alt popülasyon olarak sınıflandırılmıştır. Genotipler, bir popülasyonun moleküler çeşitliliğini göstermek amacıyla üreme araştırmaları için geliştirilebilir. IPBS moleküler markörlerinin mısır çeşitlerinde genetik ve filogenetik analizler için uygun genetik araçlar olduğu belirlenmiştir. Bu sonuca yukarıda bahsedilenlerin bir sonucu olarak ulaşılmıştır. Elde edilen veriler, gelecekte mısır genetiği alanına bilimsel bir temel ve değerli bir katkı olarak hizmet edecektir.

Molecular Characterisation of Some Corn (*Zea mays* L.) Genotypes Growing in Iğdır Province

Highlights:
- Phylogenetics
- İPBS

Keywords:
- Molecular marker
- Maize
- Zea mays L.

ABSTRACT:

Inter-primer binding site markers based on retrotransposons or iPBS retrotransposon markers have been useful in determining genetic diversity in a large number of organisms. The CTAB technique was employed to isolate DNA, and iPBS molecular markers were employed to conduct genetic diversity studies. Based on the analyses' findings, the genotypes exhibited a significant degree of genetic diversity, with a 100% polymorphic locus percentage. A total of 154 polymorphic bands were generated as a consequence of molecular identification experiments conducted with 12 IPBS markers. The typical polymorphism rate was determined to be 100%. Additionally, the average polymorphism (PIC) value, which quantifies the gene diversity of all markers examined in the study, was 0.228, and the average H value was 0.274. The genotypes 1 and 14 exhibited the lowest similarity ratio, with a coefficient value of 0.1600, when the DICE similarity coefficients were compared. These results were derived by comparing the DICE similarity coefficients of the samples. The analysis determined that the greatest similarity value between genotypes 4 and 3 was 0.6747. Maize genotypes are classified into four distinct subpopulations. Genotypes can be developed for reproductive research in order to demonstrate the molecular diversity of a population. It was determined that IPBS molecular markers are appropriate genetic instruments for genetic and phylogenetic analyses in maize varieties. This conclusion was attained as a consequence of the aforementioned. The data that is gathered will serve as a scientific foundation and a valuable contribution to the field of maize genetics in the future.

[1]Gizem ÇEVİK (**Orcid ID:** 0000-0003-2494-1852), Iğdır University, Graduate School of Education, Iğdır, Türkiye
[2]Barış EREN (**Orcid ID:** 0000-0002-3852-6476), Iğdır University, Faculty of Agriculture, Department of Agricultural Biotechnology, Iğdır, Türkiye

***Sorumlu Yazar/*Corresponding Author*:** Baris EREN, e-mail: bariseren86@gmail.com

This study was produced from Gizem ÇEVİK's Master's thesis.

INTRODUCTION

Maize is a plant that grows in warm climates and has significant economic value (Keskin et al., 2018). It is cultivated worldwide due to its excellent adaptability and wide diversity. In addition to its use in human and animal nutrition, maize has a wide range of uses as raw material in industry, and its use in biofuel production is increasing. Maize, the third most important crop in Turkey and the second most important crop in the world in terms of cultivated area, is the third most important grain crop in our country after wheat and barley (Keskin et al., 2017a). Grain maize is widely used in nutrition due to its great importance in terms of the nutrients it contains. Maize is mostly grown as a primary or secondary crop in the southern and south-western regions of Turkey. Compared to other cereals, maize is used more in industry, although its use in agricultural environments is increasing daily. It is widely used in the nutrition of both humans and animals (Keskin et al., 2018). The maize plant is constantly evolving as a result of its high unit area grain yield, many hybrid variations, breeding methods, and high yield (Keskin et al., 2017b).

To increase yield in maize cultivation, in addition to the cultural activities generally used, it is also essential to use the appropriate seed for the conditions of the region. In many parts of the world, reaching the desired yield levels is impossible because it is not possible to select the appropriate genotype that impacts cereal production (Demir, 2016). Extensive research will be required in the region to select the right variety. Today, the agricultural sector is responsible for feeding an increasing population. To overcome this responsibility and ensure sustainable food production, a deeper understanding of plant genetic resources is needed. In this context, Zea mays (maize) stands out as an important cereal and industrial crop worldwide (Şakiroğlu, 2010). Maize plays a key role in meeting nutritional needs and is a basic material for bioenergy production and industrial uses.

The DNA molecule's encoding and transmission to subsequent generations form the basis of genetic diversity. Hughes et al. (2008) state that new species emerge when there are changes in the structure of DNA. These changes in DNA, both within and between species, have an impact on the functioning of our ecosystem. Genetic diversity is essential for the adaptation of life. Variations in DNA allow organisms to adapt to new conditions and survive. There is a direct relevance here to agriculture because these variations, which arise from the coding of some important genes, confer desirable traits to offspring, such as increased seed hardiness, plant robustness, and stability (Bruford et al., 2016).

The genetic characterization of maize plays an important role in the development of plant breeding, agricultural biotechnology, and sustainable agricultural practices. Technological advances in the field of molecular biology and genetics allow us to understand the genetic structure of maize in more detail and to improve important traits of this plant, such as adaptation, disease resistance, and yield increase (Eren et al., 2023a). Compared to characterizing an organism using DNA markers, morphological characterization has a more significant number of disadvantages. For example, the environment can sometimes influence the organism's characteristics, and waiting for the entire growth period to classify is both a time-consuming and significantly more costly procedure (Jonah et al., 2011).

The polymerase chain reaction, also known as PCR (Polymerase Chain Reaction), is an enzymatic technique that analyses a specific site between two sections of known sequence in DNA. Primers are an initiator DNA technology created based on the polymerase chain reaction (PCR). These molecules, known as initiator DNA, are characterized by specific sequences and can be produced synthetically (Eren et al., 2023b). Among these methods, the most commonly used are RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphisms), SSR (simple sequence repeats), and SNP (single nucleotide polymorphism) (Bark and

Havey, 1995; Kantety et al., 1995; Hajj-Moussa et al., 1996; Cömertpay et al., 2012; Kharb, 2016; Celik and Aydin, 2023; Aydın, 2024). These methodologies determine the degree of genetic relatedness between and within species in a wide range of plant and animal species (Eren et al., 2023a).

In recent years, microsatellite markers have become more popular as they provide both more comprehensive and precise information and are considered to be relatively easy to implement (Türkoğlu et al., 2023a; Aydin, 2023). Consisting of consecutive repeats of two or more nucleotide sequences in different areas of the genome, microsatellites have the ability to transmit information about the population in both directions (Gupta and Rustgi, 2004). Breeding efforts can be guided by estimating both genetic distance and degree of homozygosity (Kemp et al., 1993). Thanks to these technologies that accelerate the breeding process, some time is required to examine and evaluate the accessible material (Remya et al., 2010). This method ensures that every aspect of the research project can be carried out in an organized manner. Universal markers are cost-effective and high-throughput and depend on tRNA's presence as a reverse transcriptase primer binding site (Kalendar et al. 2010; Nadeem et al. 2018). In contrast, retrotransposon-based inter-primer binding site (iPBS) markers have several advantages over other retrotransposon markers (Andeden et al., 2013; Baloch et al., 2015a, 2015b; Shirmohammadli et al., 2018). In agriculture, the iPBS-retrotransposon marker system has been applied in many crops (Kocak et al., 2023).

Studying the genetic structure of maize (Zea mays) using molecular characterization methods provides detailed information on the genetic diversity, gene expression and genetic regulation mechanisms of this plant, allowing the development of strategies for disease resistance, adaptation to environmental stress conditions and increasing agricultural productivity. In this process, the use of retrotransposon-based marker systems, especially iPBS (inter-priming binding site) markers, can reveal the diversity in the genetic structure of maize plants more accurately and comprehensively.

The aim of this study was to characterize the genetic structure of maize plant in detail by modern molecular biotechnology techniques, especially retrotransposon-based iPBS marker systems. The study aims to examine genetic diversity, genetic affinities and potentially important agronomic traits in maize. This characterization aims to contribute to sustainable agricultural practices by providing basic information for the development of disease resistant, resistant to environmental stresses and high yielding maize varieties. In addition, in the light of the genetic data obtained, it will contribute to making more informed decisions on the selection of suitable genotypes in the regions where maize is grown.

MATERIALS AND METHODS

The study used maize varieties cultivated in Igdir ecological conditions as plant material. The maize variety names and companies used in the study are given (Table 1).

DNA Isolation

DNA isolation was performed by modifying the CTAB (Cetyltrimethylammonium bromide) protocol of Saghai-Maroof et al. (1984). By the applied DNA isolation protocol, 100 mg leaf sample was crushed in an eppendorf tube using liquid nitrogen in a tissue disintegrator device. 900 µl CTAB buffer was added and vortexed for 1-2 min for mixing. 4 µl RNase solution and 4 µl ProteinaseK solution were added, gently mixed, and incubated at 37oC for 15 minutes. The eppendorf tubes were then kept in a water bath at 65oC for 1 hour while the eppendorf tubes were mixed every 15 minutes. After incubation, the samples were kept at room temperature for 5 min. Then, 900 µl of chloroform:isoamylalcohol (24:1) (v/v) mixture was added and mixed very slowly for 10 min to prevent damage to DNA. After centrifugation at 14,000 rpm for 15 min, the supernatant was taken into a clean ependorfa (Hossein-Pour et al., 2019; Demirel et al., 2023; Türkoğlu et al., 2023b).

Table 1. Variety and company names of maize seeds used

Genotipler	Tohum Çeşidi Firma	Tohum Çeşidi Firma
G1	30B74	Pioneer
G2	KVS	Wario
G3	P2088	Pioneer
G4	P2105	Pioneer
G5	75 MAY 75	May
G6	SYBAMBUS	Sygenta
G7	P1884	Pioneer
G8	SYGLADIUS	Sygenta
G9	P0937	Pioneer
G10	PR31Y43	Pioneer
G11	PR31698	Pioneer
G12	P1551	Pioneer
G13	P1332	Pioneer
G14	P1884	Pioneer
G15	P2085	Pioneer

The maize genotypes to be used in the research were identified by performing the following stages of marker analyses to determine the genetic diversity.

Spectrophotometric Measurement of DNA Quantity

The amounts of DNA isolated and purity determined by agarose gel electrophoresis were measured by the Qubit ® 2.0 (Fluorometer, INVITROGEN) device for double-stranded DNAs. Stock DNAs isolated according to the purity image and quality analysis were diluted to a stock concentration of 5 ng/ µl for PCR reactions.

Identification of Primers with High Reliability and Polymorphism

Although the primers available in the literature are well described and published by the researcher, it is necessary to determine reliable primers before the study. For these reasons, in the first stage, a certain number of primers were selected, and the reliability and polymorphism status of the primers were tested by amplifying the DNA of 15 genotypes representing the population and running them on agarose gel. In selecting molecular markers to be used in the preliminary study, it is important that they have high polymorphism levels, are evenly distributed on the maize genome, do not show null alleles, and are reliable and reproducible. In this study, among the selected primer pairs, the primers that were robust and formed bright bands in agarose gel in 15 genotypes and showed high polymorphism were selected and used in population molecular analyses. Polymerase Chain Reaction (PCR) components of molecular markers used in the study were 7.8 µl dH2O, 1 µl primer (0.6 mM), 1. 5 µl 10X PCR buffer (750 mM Tris-HCl pH 8.8, 200 mM (NH4)2SO4, 0.1% (v/v) Tween-20), 1.5 µl MgCl2 (25 mM), 1.5 µl dNTP (2 mM each dNTP (dATP, dGTP, dCTP and dTTP)), 0. 2 µl Taq DNA polymerase (5 U/µl) and 1.5 µl DNA (20 ng/µl) for a total volume of 15 µl and the resulting bands were visualized under ultraviolet light after agarose gel electrophoresis (Hossein-Pour et al., 2019; Demirel et al., 2024).

Agarose Gel Electrophoresis

To prepare the 1% agarose gel, agarose, 1X Tris-Acetic Acid-EDTA (TAE) buffer and SafeView™ Classic (G108, ABM) were added at the rate of 10µl per 100 ml of agarose solution for gel staining. The gel was then poured into electrophoresis cassettes, combs were attached and the gel was prepared for loading after polymerisation. Then 2 µl of the isolated samples were taken and 8 µl of ultrapure water (UP) and 2 µl of bromine phenol blue were added. This mixture was loaded into the wells using a micropipette. In the first well, 2 µl of 1kb (DNA ladder, Fermentas GeneRuler TM) was

placed. Electrophoresis was carried out for two hours at 60 Volt voltage and 400 mA current. After electrophoresis, the samples were visualised under ultraviolet (UV) light with G-box (SYNGENE, USA) imaging system and transferred to computer (Kumlay et al., 2021).

Table 2. Information about the iPBS marker used in genomic analyses

No	Primer Adı	Sekanslar 5'--3'	(°C)
1	IPBS-2074	GCTCTGATACCA	40.5
2	IPBS-2231	ACTTGGATGCTGATACCA	52.9
3	IPBS-2221	ACCTAGCTCACGATGCCA	58
4	IPBS-2273	GCTCATCATGCCA	47.6
5	IPBS-2298	AGAAGAGCTCTGATACCA	51.6
6	IPBS-2390	GCAACAACCCCA	47.6
7	IPBS-2219	GAACTTATGCCGATACCA	51.5
8	IPBS-2271	GGCTCGGATGCCA	54.3
9	IPBS-2376	TAGATGGCACCA	43.1
10	IPBS-2087	GCAATGGAACCA	43.5
11	IPBS-2077	CTCACGATGCCA	46.1
12	IPBS-2228	CATTGGCTCTTGATACCA	51.9

Molecular Data Analysis

DNA bands observed under the sonar ultraviolet after being running in agaroz gel electrophoresis after the PCR procedure were coded as "1" in case of tape, and "0" in case of lack of tape and data files were created. Polymorphism rates of molecular markers were obtained as a result of multiplying the total number of polymorphic bands obtained from primaries by dividing the total number of bands by 100. The DNA data obtained were analyzed using NTSYS (Numeric Taxonomy Multivaria Analysis System, NTSYS-PC Version 2.1, Exeter Software, Setauket, N.Y., USA) package program (Rohlf, 2000). Using this program, the similarity indexes between the populations were calculated first (Dice, 1945) and the similarity index was created with the UPGMA method.

RESULTS AND DISCUSSION

Genomic Analysis and Genetic Diversity

Molecular characterization experiments were performed using PCR-based IPBS markers on fifteen different registered corn genotypes to determine the genetic differences between Egyptian genotypes. 15 IPBS markers used in the molecular study of the Egyptian genotype found a polymorphism (difference). The band profiles produced from IPBS markers used in molecular analysis are shown in Table 3 with the diversity values of the markers.

Table 3. Characterization Results of IPBS markers in corn genotypes

			Bant Sayıları		Çeşitlilik		
No	**Primer Adı**	**ºC**	**Top. Bant**	**Polimorfik Bant**	**P %**	**H**	**PIC**
1	IPBS-2074	40.5	11	11	100	0.276	0.229
2	IPBS-2231	52.9	19	19	100	0.201	0.176
3	IPBS-2221	58	4	4	100	0.240	0.204
4	IPBS-2273	47.6	20	20	100	0.306	0.250
5	IPBS-2298	51.6	9	9	100	0.282	0.236
6	IPBS-2390	47.6	16	16	100	0.260	0.220
7	IPBS-2219	51.5	16	16	100	0.286	0.236
8	IPBS-2271	54.3	12	12	100	0.298	0.244
9	IPBS-2376	43.1	7	7	100	0.257	0.215
10	IPBS-2087	43.5	17	17	100	0.274	0.226
11	IPBS-2077	46.1	12	12	100	0.274	0.231
12	IPBS-2228	51.9	11	11	100	0.339	0.273
Toplam			154	154			
Ortalama			12.83	12.83	100	0.274	0.228

ºC: attachment temperature, P%: Polymorphism, H: Gene Diversity, PIC: Polymorphism Information Content

Among the 12 IPBS markers used, the total number of bands per marker was the lowest marker, the IPBS-2221 Marker. Marker, which has the highest number of bands, was Marker with IPBS-2273 with a 100 %polymorphism rate. On the other hand, the number of polymorphic bands was 154, and the total number of bands obtained from corn genotypes was 154. Markör 4 and IPBS-2221 marker with the minimum number of polymorphic bands. On the other hand, IPBS-2273 markers have the highest number of polymorphic bands with 20 pieces. According to the findings, the average number of polymorphic bands in each marker was 12.83.

Molecular identification was made using 12 IPBS markers, and the results showed that a total of 154 polymorphic bands were produced. The average polymorphism rate was 100 %. In addition, the average polymorphism (PIC) representing the gene diversity of all markers examined in the study was 0.228, and the average h value was 0.274. It was determined that H (gene diversity) obtained for each marker used is between 0,201-0,339. The lowest H value was obtained in the Markör with 0,201 and IPBS-2231 and the highest H in IPBS-2228 with 0.339 and 0.339.

Definition of Corn genotypes

As part of the study, molecular identification was performed on 15 corn genotypes using 12 IPBS markers. In the Excel table, every collected band is considered as '1' and '0' if unavailable. Data Analysis was performed with the help of the NTSYSPC 2.11F program. o analyze the IPBS DNA data of Egyptian gents, the DICE technique was used to calculate similarity coefficients (Fig 2). It has been found that genotypes have an average of 0.43 DICE similarity coefficients. When the genotypes of genotypes were compared with the ex -similarity coefficients, it was found that the number 1 and 14 has the lowest level of similarity with the 0.1600 coefficient value. In the comparison of genotype 4 and 3, the highest similarity coefficient was found to be 0,6747.

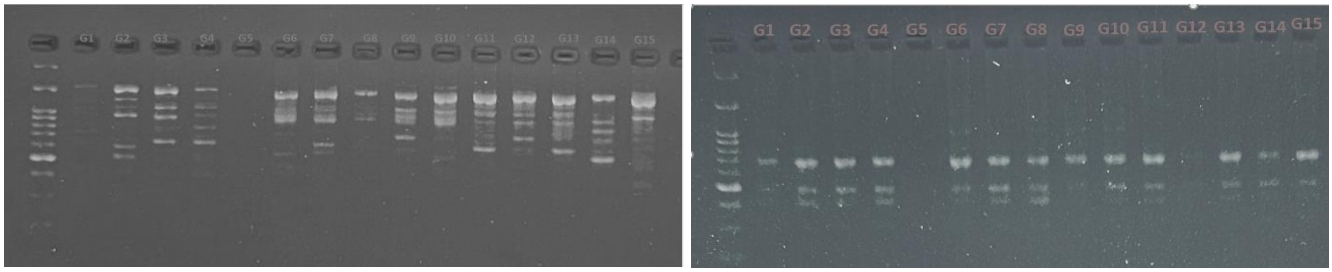

Figure 1. DNA profiles of IPBS-2273, IPBS-2376 markers

his study, a cluster analysis was performed on 15 different maize genotypes using the UPGMA technique and the DICE similarity index. The dendrogram was used as the basis for constructing an ultrametric similarity matrix using the study's findings. The Mantel test was performed using the DICE similarity matrix (Mantel, 1967). The correlation coefficient value was found to be r=0.79891 in fifteen different maize genotypes, as shown by the collected data.

Principal component analysis was performed on maize samples using NTSYS software. Principal component analysis was used to determine the genetic diversity among genotypes. As a result of the PCA (Principle Component) study, a two-dimensional graphic was created. The findings obtained from STRUCTURE software were used to visualize individual genotypes in two-dimensional space (Sakiroğlu et al., 2010). In the dendrogram, it is seen that the degree of separation between Group 1 and Group 2 is 0.29, which shows that the similarity rate between the two groups is quite low. The fact that there is a comparable situation with this situation indicates that it is located at a distant point in the three-dimensional graphic. While this situation continues, subgroup 1 is divided into subgroups, 3 and 4. It is seen that subgroup 3, which has a similarity rate of approximately 0.39, is divided into two other groups,

5 and 6, and is positioned differently from the groups showing clustering in the two-dimensional graphic. Subgroup 4 is divided into subgroups 7 and 8 among itself. Subgroups 7 and 8 are separated from each other with a similarity ratio of 0.45. When all groups are examined, it is determined that the highest clustering occurs in subgroups 7 and 8. When the dendrogram findings are compared with the PCA (Principal Component Analysis) analysis results, it is seen that the genotypes are positioned in a comparable manner, and consistent results are obtained. All Eigenvalues are calculated with the help of NTSYS software (Table 4.5). After the Principal Component analysis (PCA) is performed, the total eigenvalues of the two and three-dimensional first principal components are found to be 66.67. This is the result of the Principal Component Analysis. It can be seen that 66.67 percent of the general variance is represented by the sum of the eigenvalues of the first three main components.

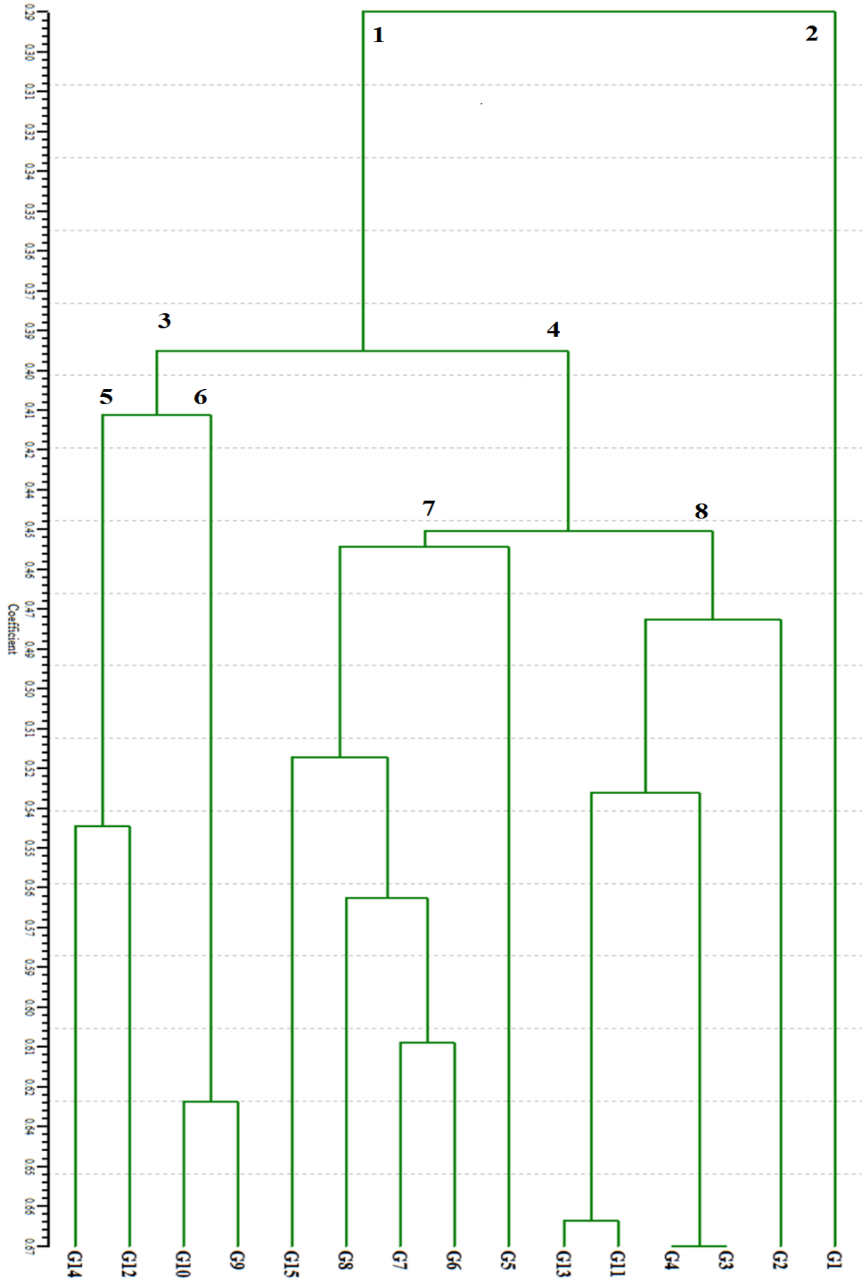

Figure 2. UPGMA dendrogram of 12 markers

Table 4. Eigen values of the first three main components

Anabileşenler	Eigen Değeri	Yüzdeleri	Eklemeli Toplamları
1.	6.97	46.52	46.52
2.	0.99	6.61	60.88
3.	0.86	5.78	66.67

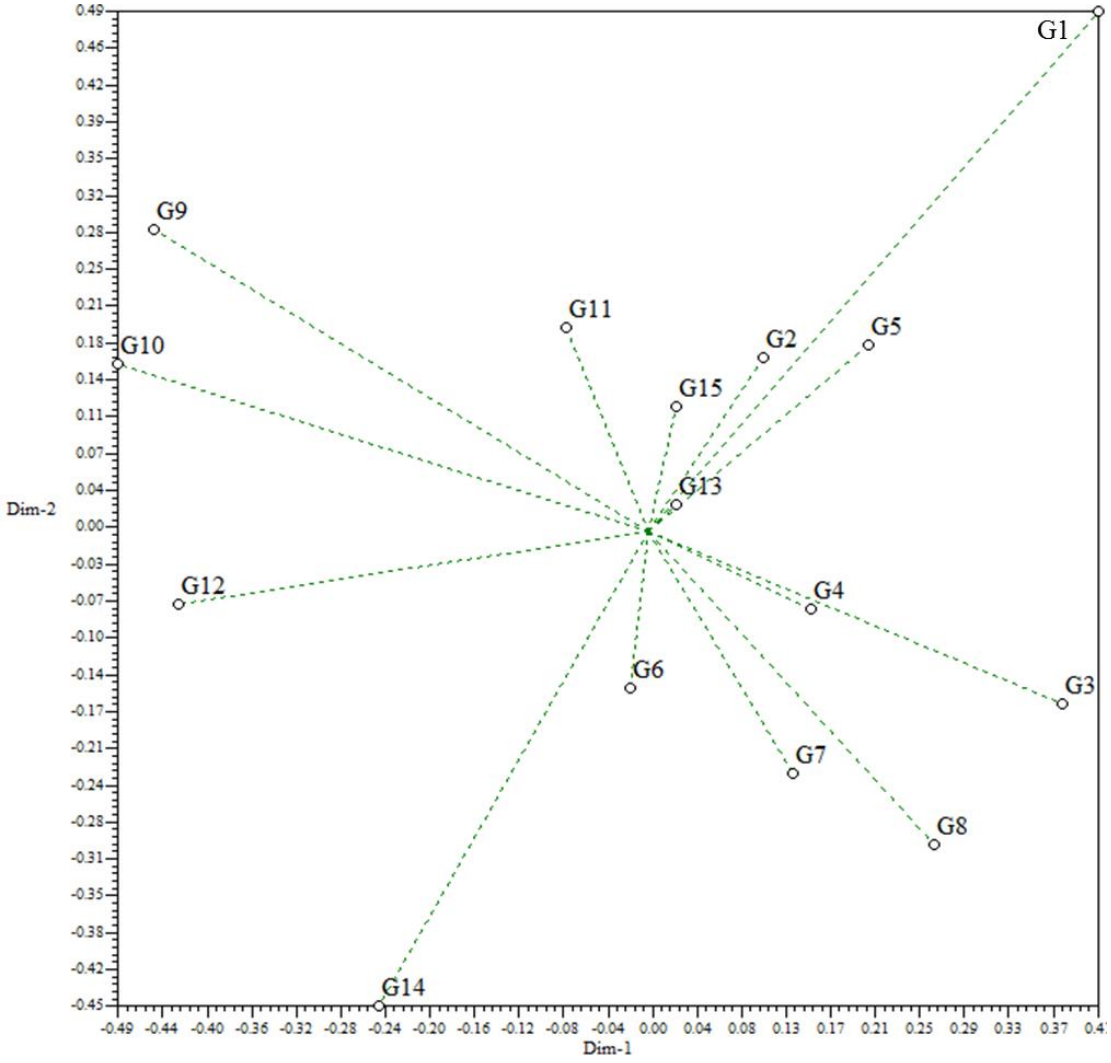

Figure 3. Two-dimensional dendrogram obtained by principal component analysis

CONCLUSION

In the molecular identification study obtained with 12 IPBS markers, a total of 154 polymorphic bands were obtained. The average polymorphic band number was determined as 100%. In the study, the average H (genetic diversity) value of all markers was obtained as 0.274 and the average polymorphism (PIC) value was 0.228. The DICE similarity coefficient of maize genotypes was determined as 0.43 on average. In addition, the correlation coefficient (r) was determined as 0.79891. When the DICE similarity coefficient of genotypes was compared, it was revealed that the lowest similarity level was between genotypes 1 and 14 with a coefficient value of 0.1600. In the comparison of genotypes 4 and 3, the highest similarity coefficient was found as 0.6747. The obtained data result was determined to be 4 subgroups and the highest clustering was in subgroups 7 and 8. Filiz et al. (2024) In their study, the highest polymorphism rate was obtained as 100% in the molecular identification made in 10 corn plants. The total number of polymorphic bands was determined as 75, and the average H value was determined

as 0.26. Baran et al. (2022) aimed to determine the genetic diversity and population structure in 32 corn genotypes in a study they conducted. While the average polymorphism rate obtained was 100%, the average PIC value was determined as 0.65. In addition, the average H genetic diversity was determined as 0.178. This study is also similar to the literature data. As a result, the collected corn varieties are of great importance in terms of plant breeding and protection of genetic resources. It is of great importance that the maintenance and evaluation of these resources are carried out in order to prepare for later breeding studies. In terms of agriculture, it is believed that the obtained data will provide new information and shed light on breeding research. In addition, important results were obtained in the process of parent selection for future breeding projects in the light of the collected molecular data. These results were achieved by considering the genomic characteristics of maize genotypes. According to one school of thought, registration of existing maize genotypes and breeding of these genotypes will make it possible to produce new maize varieties.

ACKNOWLEDGEMENTS

This study was supported by Iğdır University Scientific Research Projects unit as project number ZİF0324Y07.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

REFERENCES

Andeden, E. E., Baloch, F. S., Derya, M., Kilian, B., & Özkan, H. (2013). iPBS-Retrotransposons-based genetic diversity and relationship among wild annual Cicer species. Journal of Plant Biochemistry and Biotechnology, 22, 453-466.

Aydin, A. (2023). Determination of genetic diversity of some upland and Sea Island cotton genotypes using high-resolution capillary electrophoresis gel. Agronomy, 13(9), 2407.

Aydın, A. (2024). Determining the genetic diversity of some black cumin genotypes collected in different regions of Türkiye using RAPD markers. International Journal of Agriculture Environment and Food Sciences, 8(2), 294-300.

Baloch, F. S., Alsaleh, A., de Miera, L. E. S., Hatipoğlu, R., Çiftçi, V., Karaköy, T., ... & Özkan, H. (2015). DNA based iPBS-retrotransposon markers for investigating the population structure of pea (Pisum sativum) germplasm from Turkey. Biochemical Systematics and Ecology, 61, 244-252.

Baran, N., Nadeem, M. A., Yılmaz, A., Andırman, M., Kurt, F., Temiz, G., & Baloch, F. (2022). Exploring genetic diversity and population structure of Turkish Black Sea region maize (Zea mays L.) germplasm using SSR markers. Erzincan University Journal of Science and Technology, 15(3), 953-963.

Bark, O. H., & Havey, M. J. (1995). Similarities and relationships among populations of the bulb onion as estimated by RFLPs. Theoretical and Applied Genetics, 90, 407-414.

Bruford, M. W., Davies, N., Dulloo, M. E., Faith, D. P., & Walters, M. (2017). Monitoring changes in genetic diversity. The GEO handbook on biodiversity observation networks, 107-128.

Celik, I., & Aydin, A. (2023). SSR mining of black cumin (Nigella sativa L.) transcriptome for molecular marker development. Genetic Resources and Crop Evolution, 70(2), 629-638.

Cömertpay, G., Baloch, F. S., Kilian, B., Ülger, A. C., & Özkan, H. (2012). Diversity assessment of Turkish maize landraces based on fluorescent labelled SSR markers. Plant Molecular Biology Reporter, 30, 261-274.

Demir, E. (2016). Farklı ekolojik koşullarda bazı atdişi mısır genotiplerinin performanslarının belirlenmesi (Master's thesis, Hatay Mustafa Kemal Üniversitesi).

Demirel, F., Türkoğlu, A., Haliloğlu, K., Eren, B., Özkan, G., Uysal, P., ... & Bocianowski, J. (2023). Mammalian sex hormones as steroid-structured compounds in wheat seedling: Template of the cytosine methylation alteration and retrotransposon polymorphisms with iPBS and CRED-iBPS techniques. Applied Sciences, 13(17), 9538.

Demirel, F., Yıldırım, B., Eren, B., Demirel, S., Türkoğlu, A., Haliloğlu, K., ... & Bocianowski, J. (2024). Revealing genetic diversity and population structure in Türkiye's wheat germplasm using iPBS-retrotransposon markers. Agronomy, 14(2), 300.

Dice LR, 1945. Measures of the amount of ecological association between species. Ecology 26: 297–307.

Eren, B., Keskin, B., Demirel, F., Demirel, S., Türkoğlu, A., Yilmaz, A., & Haliloğlu, K. (2023a). Assessment of genetic diversity and population structure in local alfalfa genotypes using iPBS molecular markers. Genetic Resources and Crop Evolution, 70(2), 617-628.

Eren, B., Türkoğlu, A., Haliloğlu, K., Demirel, F., Nowosad, K., Özkan, G., ... & Bocianowski, J. (2023b). Investigation of the influence of polyamines on mature embryo culture and DNA methylation of wheat (Triticum aestivum L.) using the machine learning algorithm method. Plants, 12(18), 3261.

Fılız, E., Uras, M. E., Ozturk, N., Gungor, H., & Ozyıgıt, I. I. (2024). Genetic diversity and phylogenetic analyses of Turkish sweet corn (Zea mays var. saccharata) varieties using ISSR markers and chloroplast trnL-F IGS region. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 52(2), 13551-13551.

Gupta, P. K., & Rustgi, S. (2004). Molecular markers from the transcribed/expressed region of the genome in higher plants. Functional & Integrative Genomics, 4, 139-162.

Hajj-Moussa, E., Millán, T., Gil, J., & Cubero, J. I. (1996). Variability and genome length estimation in chickpea (Cicer arietinum L.) revealed by RAPD analysis. Journal of Genetics and Breeding, 51, 83–85.

Hossein-Pour A, Ozkan G, Nalci O, Haliloglu K (2019) estimation of genomic instability and dna methylation due to aluminum (al) stress in wheat (Triticum aestivum L.) using ipbs and cred-ipbs analyses. Turk J Bot 43:27–37.

Hughes, A. R., Inouye, B. D., Johnson, M. T., Underwood, N., & Vellend, M. (2008). Ecological consequences of genetic diversity. Ecology letters, 11(6), 609-623.

Jonah, P. M., Bello, L. L., Lucky, O., Midau, A., & Moruppa, S. M. (2011). The importance of molecular markers in plant breeding programmes. Global Journal of Science Frontier Research, 11(5), 5-12.

Kalendar, R., Antonius, K., Smýkal, P., & Schulman, A. H. (2010). iPBS: a universal method for DNA fingerprinting and retrotransposon isolation. Theoretical and Applied Genetics, 121, 1419-1430.

Kantety, R. V., Zeng, X. P., Bennetzen, J. L., & Zehr, B. E. (1995). Assessment of genetic diversity in Dent and Popcorn (Zea mays L.) inbred lines using inter-simple sequence repeat (ISSR) amplification. Molecular Breeding, 1, 365–373.

Kemp, S. J., Brezinsky, L., & Teale, A. J. (1993). A panel of bovine, ovine and caprine polymorphic microsatellites. Animal Genetics, 24, 363-365.

Keskin, B., Akdeniz, H., Temel, S., & Eren, B. (2018). Determination of feeding values of different grain corn (Zea mays L.) varieties, Atatürk Univ., J. of the Agricultural Faculty, 49 (1): 15-19

Keskin, B., Akdeniz, H., Temel, S., Eren, B. (2017b). The Yield and Yield Components of Some Silage Maize (Zea mays L.) Varieties as the Second Crop. VIII. International Scientific Agriculture Symposium, Jahorina, October 05-08, 2017, pp:1029-1036.

Keskin, B., Temel, S., & Eren, B. (2017a). Determination of yield and plant characteristics of some silage corn varieties. Journal of the Institute of Science and Technology, 7(1), 347-351.

Kharb, P. (2016). Identification of hybrids and their parents through SSR profiling in maize [PhD thesis, CCSHAU].

Kocak, M. Z., Kaysim, M. G., Aydın, A., Erdinc, C., & Kulak, M. (2023). Genetic diversity of flax genotypes (Linum usitatissimum L.) by using agro-morphological properties and molecular markers. Genetic Resources and Crop Evolution, 70(8), 2279-2306.

Kumlay, A. M., Eren, B., Demirel, S., Demirel, F., & Yıldırım, B. (2021). Bazı Pamuk Çeşitlerinde iPBS Analiziyle Genetik Varyasyonun Ortaya Çıkarılması. Avrupa Bilim ve Teknoloji Dergisi, (21), 67-73.

Mantel N, 1967. The detection of disease clustering and a generalized regression approach. Cancer Res 27: 209–220.

Nadeem, M. A., Nawaz, M. A., Shahid, M. Q., Doğan, Y., Comertpay, G., Yıldız, M., ... & Baloch, F. S. (2018). DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. Biotechnology & Biotechnological Equipment, 32(2), 261-285.

Remya, K. S., Joseph, S., Lakshmi, P. K., & Akhila, S. (2010). Microsatellites in varied arenas of research. Journal of Pharmacy and Bioallied Sciences, 2(2), 141-143.

Rohlf, F.J. (2000) NTSYS-pc: numerical taxonomy (Vitis vinifera L.) varieties using morphological data and multivariate analysis system. Exeter Software and AFLP markers. Electron J Biotechnol 6:37–45.

Saghai-Maroof, M. A., Soliman, K. M., Jorgensen, R. A., & Allard, R. (1984). Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proceedings of the National Academy of Sciences, 81(24), 8014-8018.

Şakiroğlu, M., 2010. Bitki Genetik Kaynaklarının Uluslararası Paylaşım Sorunu SETA Analiz, Sayı: 25: 3-14.

Sakiroglu, M., Doyle, J. J., & Brummer, E. C. (2010). The population genetic structure of diploid Medicago sativa L. germplasm. In Sustainable use of genetic diversity in forage and turf breeding (pp. 143-148). Springer Netherlands.

Shirmohammadli, S., Sabouri, H., Ahangar, L., Ebadi, A. A., & Sajjadi, S. J. (2018). Genetic diversity and association analysis of rice genotypes for grain physical quality using iPBS, IRAP, and ISSR markers. Journal of Genetic Resources, 4(2), 122-129.

Türkoğlu, A., Haliloğlu, K., Tosun, M., Bujak, H., Eren, B., Demirel, F., ... & Niedbała, G. (2023a). Ethyl methanesulfonate (EMS) mutagen toxicity-induced DNA damage, cytosine methylation alteration, and iPBS-retrotransposon polymorphisms in wheat (Triticum aestivum L.). Agronomy, 13(7), 1767.

Türkoğlu, A., Haliloğlu, K., Tosun, M., Szulc, P., Demirel, F., Eren, B., ... & Niedbała, G. (2023b). Sodium azide as a chemical mutagen in wheat (Triticum aestivum L.): patterns of the genetic and epigenetic effects with iPBS and CRED-iPBS techniques. Agriculture, 13(6), 1242.