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GENETIC CHARACTERIZATION AND POPULATION STRUCTURE BY iPBS MARKERS OF BOTTLE GOURD (*Lagenaria siceraria*) GENOTYPES

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ABSTRACT

Bottle gourd (*Lagenaria siceraria* (Molina) Standl.) is a significant cucurbit plant species whose ripe fruits are consumed as ornamental items and immature fruits are consumed as vegetables. In addition, these plants are used as rootstocks in vegetable grafts due to their strong root structure. This study aimed to determine the genetic diversity of bottle gourd genotypes whose fresh fruits are consumed. Using iPBS (Inter-Primer Binding Site) retrotransposon marker techniques, a total of 186 bands in 30 genotypes were obtained and the polymorphism rate was calculated as 93.3%. Similarity coefficient values were found in the range of 0.35-0.96. Three main clusters emerged from cluster analysis. As a result of the structure analysis, genotypes were divided into four subpopulations. There was also a high level of genetic diversity among bottle gourd genotypes. To improve a variety's traits, genetic diversity can be used. The iPBS technique is also a powerful tool for understanding the genetic characterization of bottle gourd. These data can be used in breeding strategies to improve bottle gourd varieties.

Keywords: Bottle Gourd, iPBS, *Laneraria siceraria*, molecular characterization, vegetable

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INTRODUCTION

The bottle gourd (*L. siceraria*) was one of the first plants cultivated for human consumption (Schlumbaum and Vandorpe, 2012). The immature fruits of the bottle gourd are consumed as vegetables but the dried and hollowed-out ripe fruits are used to make jars, musical instruments, net holders, and decorative items. As a vegetable, bottle gourd fruits are rich in minerals, such as iron, phosphorus, potassium, calcium, and magnesium, as well as essential macronutrients, such as vitamins B, C, and E, carbohydrates, and dietary fiber (Sithole *et al*., 2015; Attar *et al*., 2019). The plant species also contains cucurbitacins, sterols, oleanolic acid, triterpenoids, tannins, saponins, flavone C-glycosides, and flavonoids (Gangwal *et al*., 2010; Dhiman *et al*., 2012). Bottle gourd fruits have different names and consumption methods in different regions of Türkiye. Bottle gourds, with their different local names (Sıyırma, Haylan, Et kabağı) are consumed as vegetables in the southern parts of Turkiye. Due to different consumption options, a type-specific cultivation is carried out by the producers. Immature fruits are offered for sale on public markets during a wide production season (from June to September). In spite of this, being an open-pollinated species may result in the lack of typical vegetable characteristics.

The bottle gourd has 2n=2x=22 chromosomes and a genome size of approximately 334 Mb (Achigan-Dako *et al*., 2008). There is little information available about the bottle gourd genome, despite its small size. In horticultural plants, molecular markers can be used for identifying cultivars, phylogenetic analyses, determining genetic relationships, marker-assisted selection, and quantitative trait locus (QTL) analyses. In eukaryotic genomes, retrotransposons constitute the largest portion of transposons and have the most genes (Sabot and Schulman 2006). Retrotransposons may accumulate differently on plant genomes, resulting in different genome sizes. Genetic diversity and phylogenetic studies used retrotransposon-based markers (Kalender *et al*., 2010; Coskun, 2023). Although some studies have been conducted to determine the molecular characterization of bottle gourd genotypes (Decker-Walters *et al*., 2001; Xu *et al*., 2011; Gurcan *et al*., 2015; Mashilo *et al*., 2016), they are not sufficient and the iPBS marker technique has not been used. The aim of this study was to determine the population structure and genetic diversity of bottle gourd genotypes.

MATERIALS AND METHODS

Material: A total of 30 bottle gourd genotypes, which are used as vegetables, were collected in Hatay, Gaziantep, Kilis, Kahramanmaraş and surrounding provinces in

Türkiye. The seeds of all genotypes of bottle gourd were planted in pots under controlled greenhouse conditions. First true leaves of genotypes were harvested and delivered to the laboratory via cold chain for DNA isolation.

Molecular study: Molecular characterization studies were carried out in the Genetics Laboratory within the Department of Horticulture, Faculty of Agriculture, Hatay Mustafa Kemal University. The first true leaves of bottle gourd genotypes were used to isolate DNA using the Cetyltriethylammnonium bromide (CTAB) method. PCR components were prepared using 30 ng Template DNA, 1U Taq DNA polymerase enzyme, 0.25 mM each dNTP, 1 µM primer, 1.5 µl 10X PCR buffer, 1.5 mM MgCl2 and ddH2O water as a PCR mixture. The PCR thermal cycling profile is as follows: initial denaturation for 3 minutes at 95 °C, 38 cycles at 95 °C for 60 s, 50-60 °C for 60 s, 72 °C for 120 s, and final extension for 10 minutes at 72 °C. The PCR products were separated on an agarose gel for 5 hours at 110V and visualized under UV light. Nineteen iPBS primers that gave acceptable results were used in PCR studies of all samples. The analysis of PCR products was carried out in 05X TBE solution on 1.5% agarose gels stained with ethidium bromide. In the UV gel imaging system, agarose gels were recorded and 1, 0 and 9 data were obtained.

Statistic Analyses: There was a determination of the number of polymorphic bands (PBS), the number of total bands (TBS) and the polymorphism rate for each primer combination. NTSYS (Numerical Taxonomy Multivariate Analysis System, NTSYSpc version 21, Exeter Software, Setauket, N.Y., USA) was used for clustering (Rohlf 2000). Similarity indices were calculated according to the Dice method and dendrograms were created according to the UPGMA (Unweighted Pair-Group Method With Arithmetic Average) method. Population structure was determined using the STRUCTURE program. Also using GenAlex (Peakall and Smouse, 2006), polymorphic information content, expected heterozygosity, unbiased expected heterozygosity, effective allele number and Shannon information index values were determined..

RESULTS

Nineteen iPBS primers were used for genetic characterization in 30 gourd genotypes. There were 186 bands obtained from the primers, with an average of 9.8 bands per primer. A total of 173 polymorphic bands and an average of 9.1 polymorphic bands per primer were obtained. The total number of bands in the primers varied between 6-14, and the highest number of bands was obtained from the iPBS-2387 and iPBS-2393 primers. The number of polymorphic bands in the primers was between 5-13, and the highest polymorphic band was obtained from the iPBS-2387 primer. The polymorphism rate varied between 55.6-100% and the average was 93.3%. The primers showing the highest polymorphism were iPBS-2376, iPBS-2383, iPBS-2272, iPBS-2277, iPBS-2217, iPBS-2243, iPBS-2249, iPBS-2251, iPBS-2252 and iPBS-2244 (100%) and the primer showing low polymorphism was iPBS-2392 (55.6%). All primers showed a band gap between 90-1480 (Table 1).

The UPGMA dendrogram showed three main clusters. Twenty genotypes were found in Cluster A, three genotypes in Cluster B (genotypes 5, 6 and 7), and seven genotypes in Cluster C (genotypes 14, 19, 20, 22, 25, 26 and 28). In terms of genotype clustering, genotypes 1 and 2 were the closest. In general, it can be said that there is a similarity between bottle gourd genotypes' regions of origin and their genetic distances. All ten Hatay genotypes were included in cluster A. However, there are ten more genotypes from other regions in cluster A. Kahramanmaraş origin bottle gourd genotype members were included in all three main groups. While 3 of the Kahramanmaraş genotypes (5, 6 and 7) constituted the entire cluster B, genotype number 15 was located in cluster A, and genotype numbers 19, 20, 22, 25, 26 and 28 were located in cluster C. All genotypes originating from Kilis province are in cluster A and close to each other. Osmaniye genotypes all belong to cluster A. While genotype numbers 11 and 13 originating from Osmaniye are genetically close to each other, genotype number 12 is located further away from the others. All but one of the Gaziantep genotypes (genotype number 14 in Cluster C) were located in cluster A (Figure 1).

For bottle gourd genotypes, two-dimensional PCA graphics were obtained using the NTSYS program. Four main clusters emerged from the two-dimensional principal component analysis graph obtained using the NTSYS program. There are 12 genotypes (2, 4, 8, 9, 10, 13, 15, 17, 18, 23 and 29) in the first cluster, 4 genotypes (11, 21, 24 and 27) in the second cluster, 5 genotypes (3, 12, 14, 16 and 30) in the third cluster and 8 genotypes (26, 7, 19, 20, 22, 25, 26 and 28) in the fourth cluster. Genotype number 5 clustered separately from other genotypes (Figure 2).

Considering the K values obtained from iPBS data using the Structure Harvester program, it was determined that 30 gourd genotypes consisted of 4 subpopulations (Figure 3). Especially in genotypes originating from Hatay, a mixed-type genetic structure is more common. While two of the genotypes originating from Hatay are in the 2nd subpopulation, one in the 3rd subpopulation and two in the 4th subpopulation, five have a mixed-type structure. Genotypes originating from Kahramanmaraş are mostly grouped under the 1st subpopulation. The 8 of the 10 genotypes originating from Kahramanmaraş are in the 1st subpopulation. While one of the other two genotypes is in the 3rd

subpopulation, the other has a mixed-type genetic structure. All genotypes originating from Kilis are located in the 3rd subpopulation. Two genotypes originating from Osmaniye are in the 3rd subpopulation, and one genotype has a mixed-type genetic structure. Two of the genotypes originating from Gaziantep were detected in the 4th subpopulation.

Table 1. Band profiles obtained using iPBS primers in bottle gourd genotypes

Primer Name	Base Sequence 5'-3'	Total Number of Bands	Number of Polymorphic Bands	Poly. Rate (%)	Band Sizes (bp)
iPBS-2074	GCTCTGATACCA	11	10	90.9	150-1090
iPBS-2375	TCGCATCAACCA	9	8	88.9	220-940
iPBS-2376	TAGATGGCACCA	11	11	100	250-1160
iPBS-2381	GTCCATCTTCCA	12	11	91.7	350-1120
iPBS-2383	GCATGGCCTCCA	12	12	100	200-1350
iPBS-2387	GCGCAATACCCA	14	13	92.9	200-1480
iPBS-2393	TACGGTACGCCA	14	12	85.7	240-1400
iPBS-2272	GGCTCAGATGCCA	8	8	100	250-1110
iPBS-2277	GGCGATGATACCA	10	10	100	100-960
iPBS-2217	ACTTGGATGTCGATACCA	7	7	100	150-1160
iPBS-2230	TCTAGGCGTCTGATACCA	10	9	90	250-1250
iPBS-2232	AGAGAGGCTCGGATACCA	9	8	88.9	200-1000
iPBS-2239	ACCTAGGCTCGGATGCCA	8	7	87.5	100-1140
iPBS-2243	AGTCAGGCTCTGTTACCA	11	11	100	90-1220
iPBS-2249	AACCGACCTCTGATACCA	10	10	100	150-1110
iPBS-2251	GAACAGGCGATGATACCA	7	7	100	180-950
iPBS-2252	TCATGGCTCATGATACCA	6	6	100	250-1150
iPBS-2392	TAGATGGTGCCA	9	5	55.6	210-1330
iPBS-2244	GGAAGGCTCTGATTACCA	8	8	100	350-1240
Total		186	173	1772	
Mean		9.8	9.1	93.3	

According to iPBS analysis, there are 1.41 effective alleles on average. This data is between 1.22 (iPBS-2381) and 1.81 (iPBS-2251). Shannon's information index was between 0.27 (iPBS-2381 and iPBS-2277) and 0.63 (iPBS-2251) (average 0.41). Expected heterozygosity values range from 0.15 (iPBS-2381) to 0.44 (iPBS-2251) (mean 0.26). Unbiased expected heterozygosity values ranged from 0.16 (iPBS-2381) to 0.46 (iPBS-2251) (mean 0.27). Polymorphic information content was determined between 0.47 (iPBS-2392) and 0.98 (iPBS-2272) (average 0.80). There is one primer (iPBS-2392) with a polymorphic information amount below 0.5 (Table 2).

Table 2. Allele frequency (p-q), Number of effective alleles (Ne), Shannon's information index (I), Expected (He) and Unbiased expected heterozygosity (uHe), Polymorphic information amount (PIC)

Primer Name	p	Q	Ne	I	He	uHe	PIC
iPBS-2074	0.46	0.54	1.68	0.56	0.38	0.40	0.73
iPBS-2375	0.55	0.45	1.40	0.41	0.25	0.26	0.58
iPBS-2376	0.15	0.85	1.35	0.38	0.23	0.24	0.96
iPBS-2381	0.18	0.82	1.22	0.27	0.15	0.16	0.90
iPBS-2383	0.25	0.75	1.50	0.46	0.30	0.31	0.90
iPBS-2387	0.30	0.70	1.35	0.41	0.25	0.26	0.83
iPBS-2393	0.35	0.65	1.40	0.43	0.27	0.28	0.79
iPBS-2272	0.13	0.87	1.30	0.35	0.21	0.22	0.98
iPBS-2277	0.17	0.83	1.24	0.27	0.16	0.17	0.91
iPBS-2217	0.35	0.65	1.68	0.58	0.39	0.41	0.85
iPBS-2230	0.20	0.80	1.28	0.32	0.19	0.19	0.89
iPBS-2232	0.43	0.57	1.32	0.34	0.21	0.22	0.68
iPBS-2239	0.48	0.52	1.44	0.42	0.27	0.28	0.65
iPBS-2243	0.27	0.73	1.39	0.43	0.26	0.27	0.85
iPBS-2249	0.12	0.88	1.27	0.31	0.18	0.19	0.97
iPBS-2251	0.43	0.57	1.81	0.63	0.44	0.46	0.79
iPBS-2252	0.62	0.38	1.59	0.52	0.35	0.36	0.56
iPBS-2392	0.63	0.37	1.29	0.31	0.19	0.20	0.47
iPBS-2244	0.23	0.77	1.33	0.38	0.23	0.24	0.88
Mean	0.33	0.67	1.41	0.41	0.26	0.27	0.80

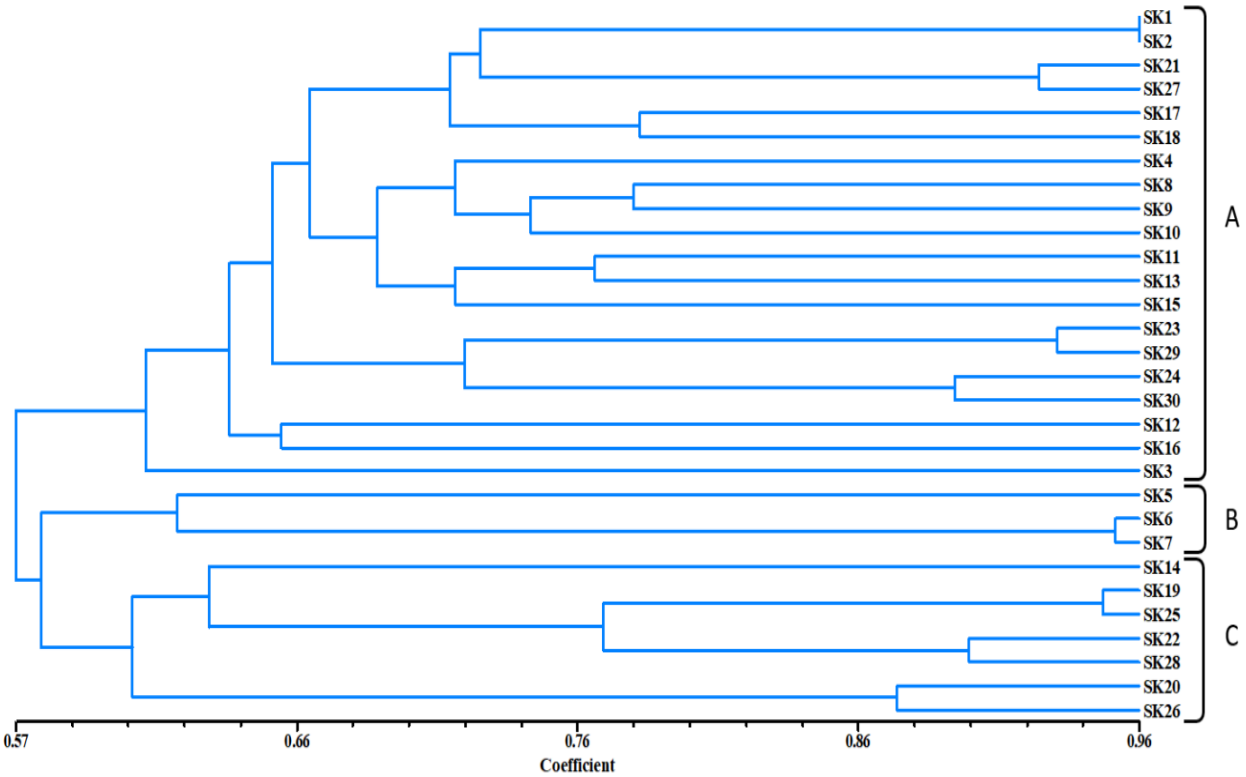

Figure 1. UPGMA dendrogram was created using the DICE similarity index in 30 bottle gourd genotypes with iPBS primers.

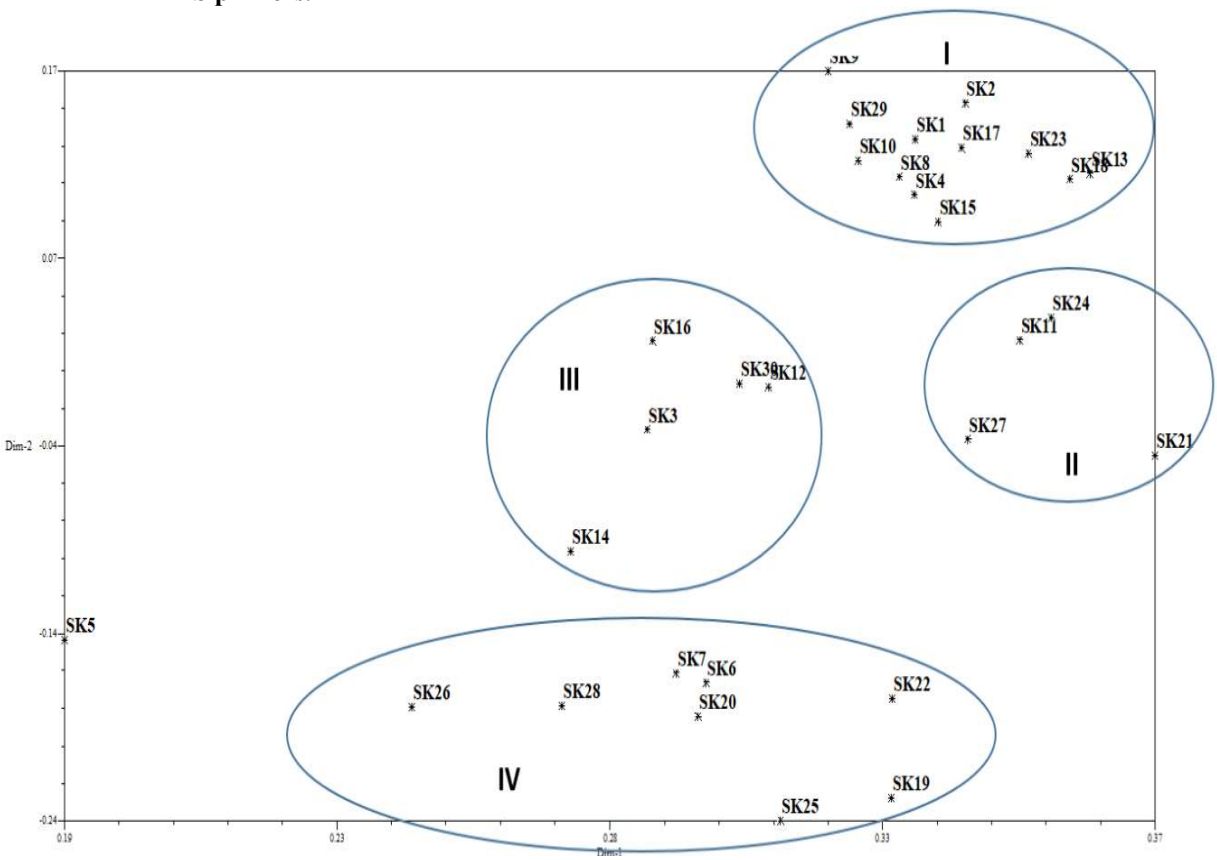

Figure 2. The two-dimensional graph obtained as a result of principal components analysis with iPBS data.

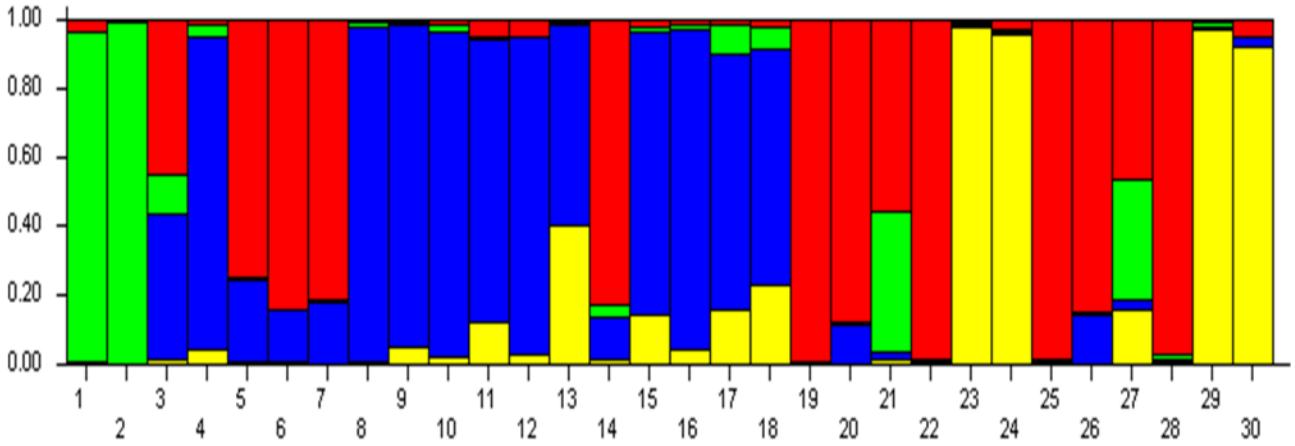

Figure 3. Graphical representation of membership coefficients in the STRUCTURE program.

DISCUSSION

Bottle gourd resources can be used more effectively in breeding studies by using different genomic tools. The use of genomic tools can also provide insight into the genetic basis of traits, enabling breeding strategies to be improved and understanding of complex traits to be better understood. In recent years, significant successes have been achieved in the development of bottle gourd genetic resources. A variety of marker-assisted selection strategies have been identified using these resources. Improvements in yield and resistance to pests and diseases have led to improved productivity and food security with bottle gourd varieties. For bottle gourd cultivation, genetic diversity is crucial, as it allows breeders to select varieties most suitable for their region. For this purpose, different marker techniques have been used.

Studies on bottle gourd genetic diversity are not sufficient (Yıldız *et al*., 2015; Gurcan *et al*., 2015). Gürcan *et al*. (2015) conducted a genetic characterization study on a total of 91 genotypes, 60 of which were of Türkiye origin. In this study, where the SSR marker technique was used, the genetic diversity value was low (0.13). In our study, it was concluded that the genetic diversity value was higher than those determined in previous studies.. Yıldız *et al*. (2015) used 16 SRAP and 40 SSR primers in 30 bottle gourds genotypes. 31 more cucurbit species were included in the study and a total of 453 bands in 61 genotypes were obtained. In this study, the average PIC value was determined as 0.15. The PIC value determined in our study is 0.80. The biggest reason why our findings differ from previous studies may be the different characteristics of the genotypes collected. In addition, the use of different marker techniques is a significant factor that creates the difference. Different marker techniques have also been used to understand genetic diversity in bottle gourd populations in other regions than Türkiye genotypes. RAPD in a study conducted in the USA (Decker-Walters *et al*., 2001), SSR in a study conducted in China (Xu *et al*., 2011), and SSR in a study carried out in North Africa (Mashilo *et al*., 2016) marker techniques were used. In some studies conducted in India, SSR (Bhawna *et al*., 2015) and ISSR (Bhawna *et al*., 2014) techniques were used.

In this study, the iPBS marker technique was studied for the first time in bottle gourds and its effectiveness was determined. In this study, a total of 186 bands were obtained from 19 primers, with an average of 9.8 bands per primer. This number is higher than SSR studies conducted on bottle gourds (Xu *et al*., 2011; Gurcan *et al*., 2015). This study also has a high polymorphism rate (93.3%). This value is higher than different marker techniques such as AFLP, RAPD and ISSR, which were previously studied in bottle gourds (Achigan-Dako, 2008; Bhawna *et al*., 2014; Kumar *et al*., 2023). The reason for this difference may be the difference in population structure and marker technique. Overall, most of the markers exhibited PIC values >0.5, indicating the high discriminatory ability of iPBS markers for analyzing bottle gourd genotypes. The results obtained show that the iPBS technique, used for the first time in gourds, has high discrimination ability in bottle gourds. As a result of this marker technique, it was determined that bottle gourd genotypes had genetic diversity. In addition, the population structure did not change according to geographical origin. Agricultural applications could benefit from this technique, which can be used to identify different bottle gourd genotypes..

Conclusıon: In this study, bottle gourd genotypes were molecularly characterized. Based on iPBS marker analysis, it was determined that thirty gourd genotypes were divided into three main clusters, and the number of subpopulations was four. These results indicate that there is a moderate level of genetic variation in gourd and that the iPBS primer technique has greater discriminatory power than other techniques. This study provides

important information to breeders to improve the characteristics of new varieties of bottle gourd in breeding programs.

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REFERENCES

Achigan-Dako, E. G., J. Fuchs, A. Ahanchede and F. R. Blattner (2008). Flow cytometric analysis in *Lagenaria siceraria* (Cucurbitaceae) indicates the correlation of genome size with usage types and growing elevation. plant systematics and evolution, 276(1-2):9-19. DOI: 10.1007/s00606-008-0075-2

Attar, U. A. and S. G. Ghane (2019). In vitro antioxidant, antidiabetic, antiacetylcholine esterase, anticancer activities and RP-HPLC analysis of phenolics from the wild bottle gourd (*Lagenaria siceraria* (Molina) Standl.). South African J. Botany, 125:360-370. DOI: 10.1016/j.sajb.2019.08.004

Bhawna, A. M. Z., L. Arya, D. Saha, A. K. Sureja, C. Pandey and M. Verma (2014). Population structure and genetic diversity in bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] germplasm from India assessed by ISSR Markers. Plant Systematics and Evolution, 300:767-773. DOI: 10.1007/s00606-014-1000-5

Bhawna, A. M. Z., L. Arya and M. Verma (2015). Transferability of cucumber microsatellite markers used for phylogenetic analysis and population structure study in bottle gourd (*Lagenaria siceraria* (Mol.) Standl.). Applied Biochemistry and Biotechnology, 175:2206-2223. DOI: 10.1007/s12010-014-1395-z

Coskun, O. F. (2023). Molecular characterization population structure analysis and association mapping of Turkish parsley genotypes using iPBS markers. Horticulturae, 9(336):1-12. DOI: 10.3390/horticulturae9030336

Decker-Walters, D., J. Staub, A. López-Sesé and E. Nakata (2001). Diversity in landraces and cultivars of bottle gourd (*Lagenaria siceraria*; Cucurbitaceae) as assessed by random amplified polymorphic DNA. Genetic Resources and Crop Evolution, 48:369-380. DOI: 10.1023/A:1012079323399

Dhiman, K., A. Gupta, D. K. Sharma, N. S. Gill and A. Goyal (2012). A review on medicinal important plants of the family Cucurbitaceae. Asian J. Clinical Nutrition, 4(1):16-26. DOI: 10.3923/ajcn.2012.16.26

Gangwal, A., S. K. Parmar and N. R. Sheth (2010). Triterpenoid, flavonoids and sterols from *Lagenaria siceraria* fruits. Scholars Research Library. Der Pharmacia Letter, 2(1):307-317.

Gurcan, K., A. Say and H. Yetisir (2015). A study of genetic diversity in bottle gourd [*Lagenaria siceraria* (Molina) Standl.] population: And implication for the historical origins on bottle gourds in Turkey. Genetic Resources and Crop Evolution, 62:321-333. DOI: 10.1007/s10722-015-0224-8

Kalendar, R., K. Antonius, P. Smýkal and A.H. Schulman (2010). iPBS: a universal method for DNA fingerprinting and retrotransposon isolation. Theoretical Applied and Genetics, 121 (8): 1419–1430.

Kumar, R., R. Kumar, B. D. Prasad, J. Kumar and B. S. Bamaniya (2023). Genetic diversity of bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] genotypes of Eastern India through agro-morphological traits and ISSR markers: Implication for future breeding. Genetic Resources and Crop Evolution, DOI: 10.1007/s10722-023-01670-0

Mashilo, J., H. Shimelis and A. Odindo (2016). Genetic diversity of bottle gourd (*Lagenaria siceraria* (Molina) Standl.) landraces of South Africa assessed by morphological traits and simple sequence repeat markers. South African J. Plant and Soil, 33:113-124. DOI: 10.1080/02571862.2015.1090024

Peakall, R., and P.E. Smouse (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes, 6: 288-295.

Rohlf, J.F (2000). NTSYS-pc: Numerical Taxonomy And Multivariate Analysis System. Exeter Software, Setauket, New York.

Sabot, F. and A.H. Schulman (2006). Parasitism and the retrotransposon life cycle in plants: Hitchhik-26 er‟s guide to the genome. Heredity, 97: 381–388.

Saxena, S., A. Singh, S. Archak, T. K. Behera, J. K. John, S. U. Meshram and A. B. Gaikwad (2014). Development of novel simple sequence repeat markers in bitter gourd (*Momordica charantia* L.) through enriched genomic libraries and their utilization in analysis of genetic diversity and cross-species transferability. Applied Biochemistry and Biotechnology, 175:93-118. DOI: 10.1007/s12010-014-1249-8

Schlumbaum, A. and P. Vandorpe (2012). A short history of *Lagenaria siceraria* (Bottle gourd) in the Roman provinces: Morphotypes and archaeogenetics. Vegetation History and

Archaeobotany, 21:499-509. DOI: 10.1007/s00334-011-0343-x

Sithole, J. N., A. T. Modi and K. Pillay (2015). An assessment of minerals and protein contents in selected South African bottle gourd landraces [*Lagenaria siceraria* (Mol.) Standl.]. Human Ecology, 51:279-286. DOI: 10.1080/09709274.2015.11906923

Xu, P., X. Wu, J. Luo, B. Wang, Y. Liu, J. D. Ehlers, Z. Lu and G. Li (2011). Partial sequencing of the bottle gourd genome reveals markers useful for phylogenetic analysis and breeding. BMC Genomics, 12:467-417. DOI: 10.1186/1471-2164-12-467

Yildiz, M., H. E. Cuevas, S. Sensoy, C. Erdinc and F. S. Baloch (2015). Transferability of Cucurbita SSR markers for genetic diversity assessment of Turkish bottle gourd (*Lagenaria siceraria*). Biochemical Systematics and Ecology, 59:45-53. DOI: 10.1016/j.bse.2015.01.006.IBT.