







## Article

# Genetic Diversity and Population Structure in Bread Wheat Germplasm from Türkiye Using iPBS-Retrotransposons-Based Markers

Kamil Haliloğlu <sup>1</sup>, Aras Türkoğlu <sup>2,\*</sup>, Ali Öztürk <sup>1</sup>, Gniewko Niedbala <sup>3,\*</sup>, Mohsen Niazian <sup>4,\*</sup>, Tomasz Wojciechowski <sup>3</sup> and Magdalena Piekutowska <sup>5</sup>

<sup>1</sup> Department of Field Crops, Faculty of Agriculture, Ataturk University, 25240 Erzurum, Turkey

<sup>2</sup> Department of Field Crops, Faculty of Agriculture, Necmettin Erbakan University, 42310 Konya, Turkey

<sup>3</sup> Department of Biosystems Engineering, Faculty of Environmental and Mechanical Engineering, Poznań University of Life Sciences, Wojska Polskiego 50, 60-627 Poznań, Poland

<sup>4</sup> Field and Horticultural Crops Research Department, Kurdistan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Sanandaj 6616936311, Iran

<sup>5</sup> Department of Geocology and Geoinformation, Institute of Biology and Earth Sciences, Pomeranian University in Słupsk, Partyzantów 27, 76-200 Słupsk, Poland

\* Correspondence: aras.turkoglu@erbakan.edu.tr (A.T.); gniewko.niedbala@up.poznan.pl (G.N.); mniazian@ut.ac.ir (M.N.)

**Abstract:** This study investigated the genetic diversity and population structure of 63 genotypes from Turkish bread wheat germplasm using iPBS-retrotransposons primers. The thirty-four iPBS primers produced a total of 1231 polymorphic bands, ranging from 8 (iPBS-2375) to 60 (iPBS-2381) alleles per marker, with an average number of 36.00 alleles. The polymorphism information content (PIC) per marker varied between 0.048 (iPBS 2087) and 0.303 (iPBS 2382), with an average of 0.175. The numbers of effective alleles ( $n_e$ ), genetic diversity of Nei ( $h$ ), and Shannon's information index (I) value were calculated as 1.157, 0.95, and 0.144, respectively. The greatest genetic distance (0.164) was between Eastern Anatolia Agricultural Research Institute genotypes and Çukurova Agricultural Research Institute genotypes. The unweighted pair-group method with arithmetic mean (UPGMA) dendrogram placed the 63 wheat genotypes into three clusters. The percentage of genetic diversity explained by each of the three main coordinates of the basic coordinate analysis was determined to be 44.58, 12.08, and 3.44, respectively. AMOVA (Analysis of Molecular Variance) showed that the variation within populations was 99% and that between populations was 1%. The result of genetic structure analysis suggests that the greatest value of K was calculated as 3. The F-statistic ( $F_{st}$ ) value was determined as 0.4005, 0.2374, and 0.3773 in the first to third subpopulations, respectively. Likewise, the expected heterozygosity values ( $H_e$ ) were determined as 0.2203, 0.2599, and 0.2155 in the first, second, and third subpopulations, respectively. According to the information obtained in the study, the most genetically distant genotypes were the G1 (Aksel 2000) and G63 (Karasu 90) genotypes. This study provided a deep insight into genetic variations in Turkish bread wheat germplasm using the iPBS-retrotransposons marker system.

**Keywords:** wheat; germplasm; genetic diversity; environmental conditions; genotypes; inter-primer binding site; varieties; PCoA; AMOVA



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## 1. Introduction

Wheat (*Triticum aestivum* L.) is hexaploid ( $2n = 6x = 42$ ) (Bhatta, 2017) and one of the most important foods in human nutrition all over the World [1]. It is also the staple diet for the majority of the global population, containing carbohydrates, protein, minerals, B-group vitamins, dietary fiber, etc. [2]. Wheat can be grown in different climatic zones ranging from the Caspian Sea to China [3]. The presumed center of wheat origin and diversity is

situated in the Fertile Crescent [4], which includes part of present-day Turkey [5]. Thus, the diversity of wheat in Turkey has a global role in providing important genetic resources for wheat improvement. In recent years, there has been a rapid increase in the number of varieties released in Turkey, and there are now 201 officially listed bread wheat varieties [6]. Turkey, particularly Southeast Anatolia, is known as the primary gene center of wheat diversification as well as the area of first wheat domestication around 10,000 BP [7–11]. Turkish wheat variations have received a great deal of attention since the beginning of the 20th century. Exploration and collection missions were mounted, and the collected germplasm was evaluated in different countries [8,12,13].

World annual wheat production was realized at 777 million tons in 2021, but world wheat demand is expected to be 1 billion tons by the year 2024 [14]. Wheat annual average production has entered a period of stagnation and declining in recent years due to biotic and abiotic stressors posing serious threats on wheat cultivation [15]. In order to use gene resources effectively in wheat breeding to produce tolerant genotypes, it is important to know the characteristics of existing gene resources and to carry out targeted breeding studies. Germplasm characterization is considered a prerequisite for breeding activities as it facilitates the introduction of novel genetic variations to the breeders that can be used for marker-assisted breeding [16].

Conventional breeding methods are largely employed to improve plant resistance against these stress factors. The process of cultivating plants has resulted in a narrowing of the genetic base in several crop types, including wheat [17]. Such a narrow genetic base poses a serious threat to sustainable production, which is very important for the rapidly increasing global population and the changing climate conditions due to global warming in recent years [18].

Genetic diversity is the basis for the long-lasting success of breeding programs, and modeling genetic diversity with various methods can reveal possible adaptations to different regions. The study of genetic variation also allows the identification of genetic characters associated with important breeding targets [19]. Different genetic materials are used in wheat breeding programs; they are used to expand the existing genetic basis [20]. Evaluation of variation in germplasm is an important step in breeding programs because it can help select varieties and lines with higher diversity and better performance under certain conditions [21,22]. Molecular markers are widely used to reveal wheat genetic diversity [18,20]. Molecular markers have become important tools for breeding studies and allow breeding studies to be concluded in a shorter time. Molecular markers are independent of environmental conditions, providing a direct estimation of genetic variation at the DNA level. Thanks to scientific studies, molecular markers with different properties have been developed over time [23,24]. Various methods have used molecular markers, including amplified fragment length polymorphisms (AFLPs) [25], random amplified polymorphic DNA (RAPD) [26], start codon targeted markers (SCoT) [27], single nucleotide polymorphism (SNP) [28], inter simple-sequence repeat (ISSR) [29], simple-sequence repeats (SSR) [30], expressed sequence tag (EST) [31], next generation sequencing (NGS) [20], diversity Arrays Technology (DArT) [32] and single nucleotide polymorphism (SNP) [33], all to assess the genetic diversity and associations among several *Triticum* species.

Retrotransposons (RTNs) are an important source of plant genetic variation, and in many plants with large genomes, over 50% of the nuclear DNA is made by retrotransposons [34]. The inter-primer binding site (iPBS) method has been verified to be an influential DNA fingerprinting technique with no need for previous knowledge of a sequence. iPBS-retrotransposons are the only retrotransposon-based marker system that has allowed polymorphism visualization throughout the plant kingdom. iPBS-retrotransposon DNA markers have been successfully utilized in assessing genetic diversity [35]. The iPBS-retrotransposon marker has been demonstrated by many studies, and there are studies on genetic diversity for these markers [16,24]. Previously, different molecular markers have been used for the molecular characterization of wheat germplasm [16]. However, there are limited studies on the characterization of wheat populations using iPBS-retrotransposons.

Therefore, the present study aimed to genetically characterize and determine the population structure of the Turkish bread wheat genotypes for the evaluation of genetic diversity. In order for breeding studies to be sustainable, it is necessary to identify, define, and use genetic resources. We believe that the findings from the study will help with the use, development, and conservation of local varieties that can adapt to changing environmental conditions.

## 2. Materials and Methods

### 2.1. Plant Materials

A total of 63 bread wheat genotypes, which were also evaluated based on early and late drought resistance under dry conditions and whose brief characteristics are presented in Table 1, were used as plant material in the study [36–38] and are available in the National Gene Bank of Turkey. Forty-three of these genotypes are included in the national cultivar list of 2007 and recommended for dry agricultural areas of Türkiye but Bezostaja 1 and Karasu 90 are recommended for irrigated agricultural areas. Twenty of them consist of registered or local varieties that have been grown or are still being grown under dry farming conditions. The plants were grown for tissue sampling in the greenhouse of Atatürk University, Department of Field Crops, Faculty of Agriculture.

**Table 1.** Some information about the bread wheat varieties used in the study.

Code Number	Genotype/Variety Name	Variety Owner Organization/Origin	Season of Sowing
Varieties included in the 2007 national variety list			
G1	Aksel 2000	Field Crops Central Research Institute.	Alternative
G2	Alparslan	Eastern Anatolia Agricultural Research Institute.	Winter
G3	Altay 2000	Anatolia Agricultural Research Institute.	Winter
G4	Athlı 2002	Field Crops Central Research Institute.	Alternative
G5	Aytın 98	Anatolia Agricultural Research Institute.	Winter
G6	Bağcı 2002	Bahri Dağdaş International Agricultural Research Institute.	Alternative
G7	Bayraktar 2000	Field Crops Central Research Institute.	Alternative
G8	Bolal 2973	Anatolia Agricultural Research Institute.	Alternative
G9	Çetinel 2000	Anatolia Agricultural Research Institute.	Winter
G10	Dağdaş 94	Bahri Dağdaş International Agricultural Research Institute.	Alternative
G11	Demir 2000	Field Crops Central Research Institute.	Alternative
G12	Doğankent 1	Çukurova Agricultural Research Institute.	Summery
G13	Doğu 88	Eastern Anatolia Agricultural Research Institute	Winter
G14	Gerek 79	Anatolia Agricultural Research Institute	Winter
G15	Gün 91	Field Crops Central Research Institute.	Winter
G16	Harmankaya 99	Anatolia Agricultural Research Institute	Winter
G17	İkizce 96	Field Crops Central Research Institute.	Alternative
G18	İzgi 2001	Anatolia Agricultural Research Institute.	Winter
G19	Karahan 99	Bahri Dağdaş International Agricultural Research Institute.	Winter
G20	Kate A-1	Trakya Agricultural Research Institute.	Winter
G21	Kıraç 66	Anatolia Agricultural Research Institute.	Winter
G22	Kırgız 95	Anatolia Agricultural Research Institute.	Winter
G23	Kırkpınar 79	Trakya Agricultural Research Institute.	Alternative
G24	Kutluk 94	Anatolia Agricultural Research Institute.	Winter
G25	Lancer	Eastern Anatolia Agricultural Research Institute.	Winter
G26	Mızrak	Field Crops Central Research Institute.	Alternative
G27	Müfitbey	Anatolia Agricultural Research Institute.	Winter
G28	Nenehatun	Eastern Anatolia Agricultural Research Institute.	Winter
G29	Palandöken 97	Eastern Anatolia Agricultural Research Institute.	Winter
G30	Pamukova 97	Sakarya Agricultural Research Institute.	Summery
G31	Pehlivan	Trakya Agricultural Research Institute.	Winter
G32	Prostor	Trakya Agricultural Research Institute.	Winter

Table 1. Cont.

Code Number	Genotype/Variety Name	Variety Owner Organization/Origin	Season of Sowing
G33	Seri 82	Çukurova Agricultural Research Institute.	Winter
G34	Soyer02	Anatolia Agricultural Research Institute.	Winter
G35	Sönmez 2001	Anatolia Agricultural Research Institute.	Winter
G36	Sultan 95	Anatolia Agricultural Research Institute.	Winter
G37	Süzen 97	Anatolia Agricultural Research Institute.	Winter
G38	Tosunbey	Field Crops Central Research Institute.	Winter
G39	Türkmen	Field Crops Central Research Institute.	Alternative
G40	Uzunayla	Field Crops Central Research Institute.	Alternative
G41	Yakar 99	Field Crops Central Research Institute.	Alternative
G42	Zencirci 2002	Field Crops Central Research Institute.	Alternative
Old cultivars and local genotypes not included in the 2007 national cultivar list			
G43	Ak-702	Anatolia Agricultural Research Institute.	Winter
G44	Ak buğday	Central Anatolia Region	Winter
G45	Ankara 093/44	Field Crops Central Research Institute.	Winter
G46	Conkesme	Eastern Anatolia Region	Alternative
G47	Haymana 79	Field Crops Central Research Institute.	Winter
G48	Kılçksız buğday	Central Anatolia Region	Winter
G49	Kırık	Eastern Anatolia Region	Alternative
G50	Kırmızı Kılçık	Eastern Anatolia Region	Alternative
G51	Kırmızı Yerli	Eastern Anatolia Region	Alternative
G52	Koca buğday	Central Anatolia Region	Winter
G53	Köse 220/39	Field Crops Central Research Institute.	Alternative
G54	Orso	Sakarya Agricultural Research Institute.	Alternative
G55	Özlu buğday	Central Anatolia Region	Winter
G56	Polatlı Kösesi	Central Anatolia Region	Alternative
G57	Sert buğday	Central Anatolia Region	Winter
G58	Sürak 1593/51	Field Crops Central Research Institute.	Winter
G59	Tir	Eastern Anatolia Region	Winter
G60	Yayla 305	Anatolia Agricultural Research Institute.	Winter
G61	Zerin	Central Anatolia Region	Alternative
G62	Bezostaja 1	Sakarya Agricultural Research Institute.	Winter
G63	Karasu 90	Eastern Anatolia Agricultural Research Institute.	Winter

## 2.2. DNA Isolation and Quantification

Young leaves of wheat (*Triticum aestivum* L.) with approximately 14-day-old plants were ground in liquid nitrogen at the molecular biology and genetics laboratory of Ataturk University. The collective DNA of 63 individuals per participation was then prepared using the DNA extraction method described in [39].

The quality of the DNA was determined by electrophoresis using agarose gel at 1% concentration. A NanoDrop ND-1000 UV/Vi's spectrophotometer device (Thermo Fisher Scientific Company, Waltham, MA, USA) was used to determine the DNA concentrations.

## 2.3. PCR and iPBS Marker Analyses

Genetic diversity analyses were performed with iPBS primers available from Sigma Aldrich (Castle Hill, NSW, Australia). In the present study, 34 iPBS primers developed by Kalendar et al. [40] were used (Table 2). PCR Amplification was performed in a thermocycler (SensoQuest Labcycler) and was conducted in a 10 µL reaction mixture comprising 25 ng template DNA, 0.5 U Taq polymerase, 0.25 mM dNTP, 1 µM (20 pmol) primer, 1X buffer, and 2 mM MgCl<sub>2</sub>. The PCR thermal cycling profile was as follows: initial denaturation for 3 min at 95 °C; 38 cycles of 95 °C for 60 s, 50–60 °C for 60 s, 72 °C for 120 s; and a final extension at 72 °C for 10 min [41]. All PCR amplification products were resolved in agarose gel at 1.5% concentration at 100 V for 105 min. Finally, gels were visualized under UV light and photographed by digital camera (Model Nikon Coolpix500) [42].

**Table 2.** List of 34 iPBS-retrotransposon primers with their sequences used to elucidate genetic diversity among 63 bread wheat genotypes.

Marker	Primers Sequences (5' → 3')	Marker	Primers Sequences (5' → 3')
iPBS-2075	CTCATGATGCCA	iPBS-2298	AGAAGAGCTCTGATACCA
iPBS-2077	CTCACGATGCCA	iPBS-2375	TCGCATCAACCA
iPBS-2079	AGGTGGGCGCCA	iPBS-2376	TAGATGGCACCA
iPBS-2080	CAGACGGCGCCA	iPBS-2377	ACGAAGGGACCA
iPBS-2087	GCAATGGAACCA	iPBS-2380	CAACCTGATCCA
iPBS-2217	ACTTGGATGTCGATACCA	iPBS-2381	GTCCATCTTCCA
iPBS-2221	ACCTAGCTCACGATGCCA	iPBS-2382	TGTTGGCTTCCA
iPBS-2225	AGCATAGCTTTGATACCA	iPBS-2383	GCATGGCCTCCA
iPBS-2228	CATTGGCTCTTGATACCA	iPBS-2384	GTAATGGGTCCA
iPBS-2231	ACTTGGATGCTGATACCA	iPBS-2385	CCATTGGGTCCA
iPBS-2242	GCCCCATGGTGGGCGCCA	iPBS-2386	CTGATCAACCCA
iPBS-2251	GAACAGGCGATGATACCA	iPBS-2387	GCGCAATACCCA
iPBS-2270	ACCTGGCGTGCCA	iPBS-2388	TTGGAAGACCCA
iPBS-2271	GGCTCGGATGCCA	iPBS-2389	ACATCCTTCCCA
iPBS-2274	ATGGTGGGCGCCA	iPBS-2390	GCAACAACCCCA
iPBS-2276	ACCTCTGATACCA	iPBS-2391	ATCTGTCAGCCA
iPBS-2278	GCTCATGATACCA	iPBS-2392	TAGATGGTGCCA

#### 2.4. Data Scoring and Analysis

The DNA bands were scored using TotalLab TL120 software (TotalLab Ltd., Gosforth, Newcastle upon Tyne, UK). For the iPBS amplification products, a band is scored “1” or absent “0” for each locus. Only clear, strong bands were scored, while faint, weak bands were ignored. The Numerical Taxonomy and Multivariate Analysis System for personal computer (NTSYSpc) v2.0 programs based on the Dice similarity matrix [43] were used to determine the genetic similarities between the varieties. A UPGMA (unweighted pair group method with arithmetic mean) dendrogram was created with the NTSYSpc V.2.0 program. In addition, molecular variance (AMOVA) and PCoA (Principal Coordinate Analysis) analyses were performed using the Genalex 6.5 program [44]. PIC (Polymorphism Information Content value) was used to assess the diversity of each iPBS marker [45]. The POPGEN v.1.32 program was used to determine the effective number of alleles ( $n_e$ ), Nei genetic diversity ( $h$ ), and Shannon’s information index ( $I$ ) [46]. The Structure v.2.3.4 program was used to determine the genetic structures of the varieties [47]. Evanno’s  $\Delta K$  [48] and Structure Harvester [49] methods were used to estimate the most expected  $K$  value. The favorable numbers of clusters ( $K$  subpopulations) were estimated (1–10) by repeating the analysis three times, according to the report of Evanno et al. [48] Using this method, Markov chain Monte Carlo (MCMC) posterior probabilities were estimated. The MCMC chains were run with a 10,000-iteration burn-in period, followed by 100,000 iterations using a model allowing for admixture and correlated allele frequencies.

### 3. Results

#### 3.1. Polymorphism Revealed by iPBS Primers

Sufficiently clear and scoreable bands were obtained from all primers included in the study. With these 34 primers, 1231 visible and scoreable bands were generated. The number of alleles in the primers varied between 8 (iPBS 2375) and 60 (iPBS 2381) (mean 36). When the analysis was performed with the iPBS markers, the PIC varied between 0.048 (iPBS 2087) and 0.303 (iPBS 2382) (mean 0.175). Major allele frequency ranged from 0.032 (iPBS-2228) to 0.888 (iPBS-2087). The mean major allele frequency was 0.193 (Table 3).



**Table 3.** Thirty-four iPBS primers used in the detection of polymorphism among 63 wheat varieties (*Triticum aestivum* L.).

Marker	Number of Alleles	Major Allele Frequency	PIC *	Marker	Number of Alleles	Major Allele Frequency	PIC *
iPBS-2075	33	0.159	0.220	iPBS-2298	50	0.063	0.187
iPBS-2077	35	0.127	0.219	iPBS-2375	8	0.444	0.220
iPBS-2079	31	0.286	0.169	iPBS-2376	16	0.413	0.131
iPBS-2080	40	0.143	0.225	iPBS-2377	39	0.159	0.223
iPBS-2087	18	0.476	0.048	iPBS-2380	47	0.190	0.279
iPBS-2217	36	0.238	0.089	iPBS-2381	60	0.032	0.279
iPBS-2221	48	0.095	0.152	iPBS-2382	32	0.206	0.303
iPBS-2225	39	0.159	0.170	iPBS-2383	35	0.159	0.128
iPBS-2228	59	0.032	0.242	iPBS-2384	37	0.254	0.128
iPBS-2231	21	0.286	0.128	iPBS-2385	48	0.063	0.146
iPBS-2242	23	0.190	0.170	iPBS-2386	31	0.286	0.058
iPBS-2251	18	0.349	0.115	iPBS-2387	47	0.095	0.160
iPBS-2270	22	0.302	0.140	iPBS-2388	41	0.111	0.178
iPBS-2271	30	0.159	0.157	iPBS-2389	38	0.143	0.187
iPBS-2274	41	0.302	0.074	iPBS-2390	26	0.206	0.123
iPBS-2276	28	0.143	0.257	iPBS-2391	51	0.127	0.262
iPBS-2278	49	0.095	0.190	iPBS-2392	54	0.063	0.207
Mean/Total					36/1231	0.193	0.175

\* PIC polymorphism information content.

### 3.2. Genetic Diversity

The number of effective alleles ( $n_e$ ), genetic diversity of Nei ( $h$ ), and Shannon's information index ( $I$ ) value of the wheat varieties are presented in Table 4. The greatest  $n_e$  (1.323),  $h$  (0.266), and  $I$  (0.182) values were observed in the iPBS-2383 primer. The lowest  $n_e$  (1.060),  $h$  (0.06), and  $I$  (0.038) values were observed in the iPBS-2087 primer. The mean  $n_e$ ,  $h$ , and  $I$  value were calculated as 1.157, 0.095, and 0.144, respectively.

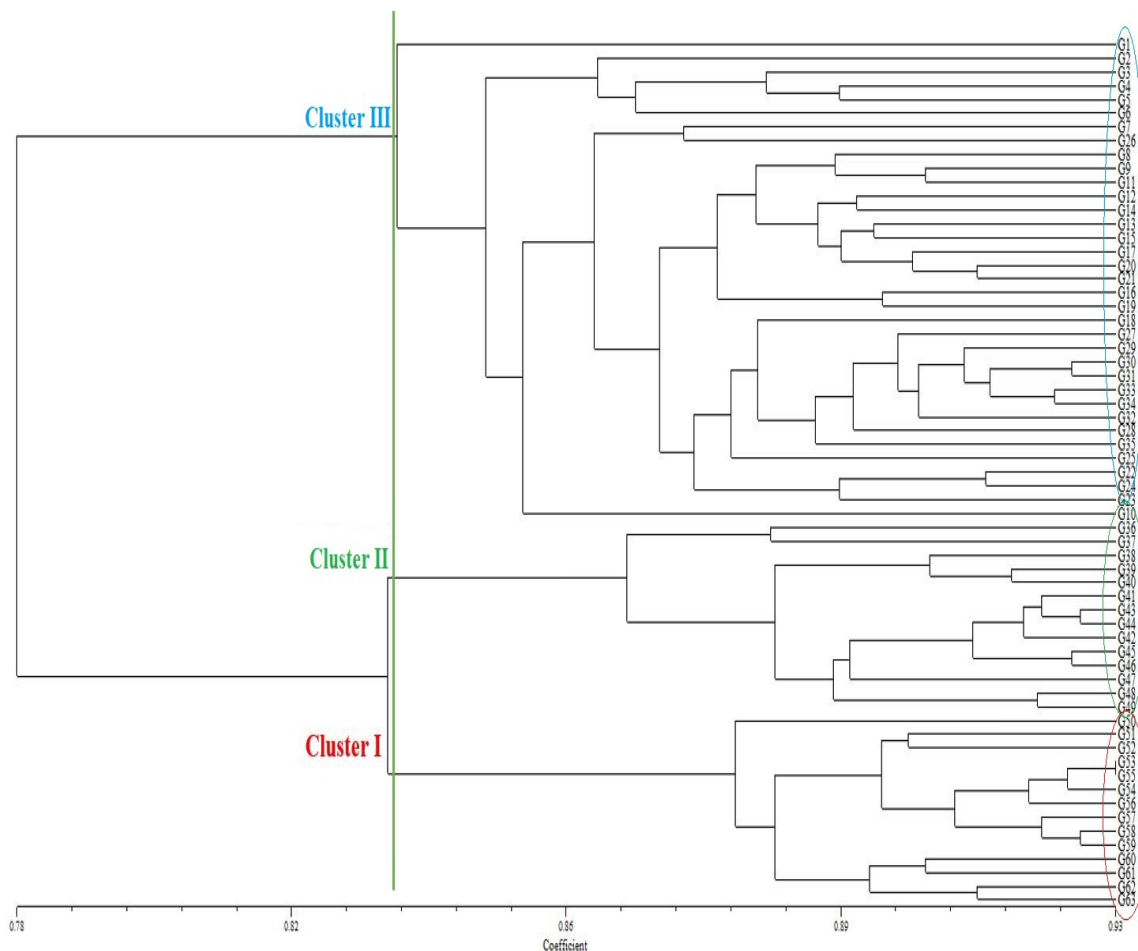
**Table 4.** Summary statistics for mean values for wheat varieties (*Triticum aestivum* L.) assessed with iPBS primers.

Marker	Ne *	H ***	I **	Marker	Ne *	H ***	I **
iPBS-2075	1.205	0.117	0.174	iPBS-2298	1.194	0.118	0.177
iPBS-2077	1.190	0.114	0.174	iPBS-2375	1.097	0.055	0.082
iPBS-2079	1.123	0.076	0.119	iPBS-2376	1.083	0.054	0.086
iPBS-2080	1.170	0.099	0.148	iPBS-2377	1.215	0.125	0.186
iPBS-2087	1.060	0.038	0.060	iPBS-2380	1.267	0.155	0.233
iPBS-2217	1.086	0.057	0.093	iPBS-2381	1.255	0.150	0.226
iPBS-2221	1.129	0.081	0.128	iPBS-2382	1.274	0.167	0.254
iPBS-2225	1.153	0.096	0.147	iPBS-2383	1.323	0.182	0.266
iPBS-2228	1.255	0.156	0.237	iPBS-2384	1.117	0.074	0.115
iPBS-2231	1.079	0.048	0.074	iPBS-2385	1.135	0.082	0.127
iPBS-2242	1.177	0.105	0.159	iPBS-2386	1.063	0.042	0.069
iPBS-2251	1.075	0.048	0.076	iPBS-2387	1.145	0.092	0.144
iPBS-2270	1.081	0.056	0.093	iPBS-2388	1.160	0.100	0.155
iPBS-2271	1.192	0.112	0.167	iPBS-2389	1.140	0.083	0.124
iPBS-2274	1.072	0.049	0.080	iPBS-2390	1.109	0.066	0.101
iPBS-2276	1.218	0.120	0.175	iPBS-2391	1.182	0.107	0.162
iPBS-2278	1.158	0.097	0.150	iPBS-2392	1.161	0.097	0.149
Mean/Total					1.157	0.095	0.144

\*  $N_e$  effective numbers of alleles, \*\*  $I$  Shannon's information index, and \*\*\*  $H_e$  gene diversity.

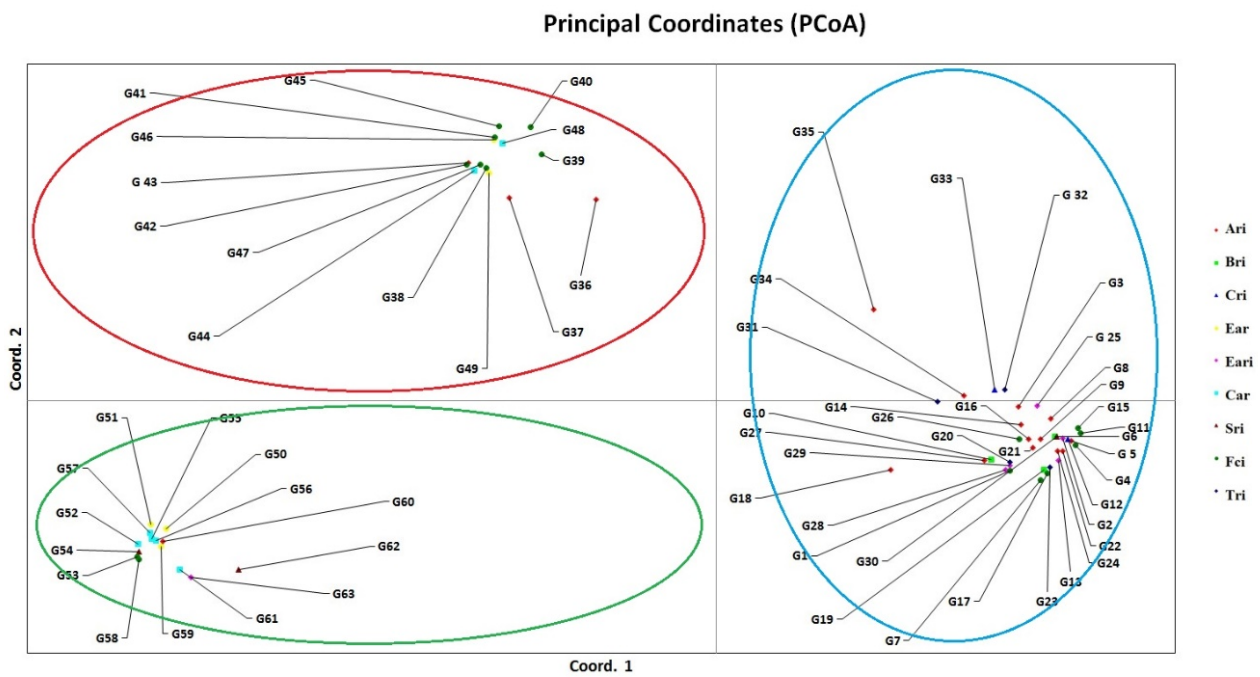
### 3.3. Cluster Analysis, Principal Coordinate Analysis (PCoA) and Genetic Distance for iPBS-Retrotransposon Markers

The unweighted pair-group method with arithmetic mean (UPGMA) dendrogram placed the 63 wheat genotypes into three clusters. Clusters I, II, and III included 14 (22.25%), 14 (22.25%), and 35 (55.5%) genotypes, respectively. (Figure 1). Cluster I contained 14 wheat genotypes, including G50, G51, G52, G53, G55, G54, G56, G57, G58, G59, G60, G61, G62, and G63. Cluster II contained 14 wheat genotypes, including G36, G37, G38, G39, G40, G41, G43, G44, G42, G45, G46, G47, G48, and G49. In addition, Cluster III contained 35 wheat genotypes, including G1, G2, G3, G4, G5, G6, G7, G26, G8, G9, G11, G12, G14, G13, G15, G17, G20, G21, G26, G19, G18, G27, G29, G30, G31, G33, G34, G32, G28, G35, G25, G22, G24, G23, and G10.



**Figure 1.** Dendrogram of 63 wheat genotypes generated with data from 34 inter-Primer Binding Site (iPBS) primers.

Principal coordinate analysis (PCoA) revealed the relative h values between the varieties, revealing three distinct groups. The result showed that the grouping pattern of the PCoA analysis corresponded with cluster analysis (Figure 2). The percentage of genetic diversity explained by each of the three main coordinates of the basic coordinate analysis was determined as 44.58, 12.08, and 3.44, respectively, and these first three components explained 60.10% of the variation (Table 5). AMOVA (Analysis of Molecular Variance) was used to detect the total variation and showed that the variation within populations was 99% and that between populations was 1% (Table 6).



**Figure 2.** Principal coordinates analysis (PCoA) calculated from the pooled data of thirty-four inter-Primer Binding Site (iPBS) primers in 63 wheat varieties. Ari: Anatolia Agricultural Research Institute, Bri: Bahri Dağdaş International Agricultural Research Institute, Cri: Çukurova Agricultural Research Institute, Ear: Eastern Anatolia Region, Eari: Eastern Anatolia Agricultural Research Institute, Car: Central Anatolia Region, Sri: Sakarya Agricultural Research Institute, Fci: Field Crops Central Research Institute, Tri: Trakya Agricultural Research Institute.

**Table 5.** PCoA analysis of wheat varieties.

Axis	1	2	3
%	44.58	12.08	3.44
Cum %	44.58	56.65	60.10

**Table 6.** AMOVA of wheat varieties using inter-Primer Binding Site (iPBS) marker.

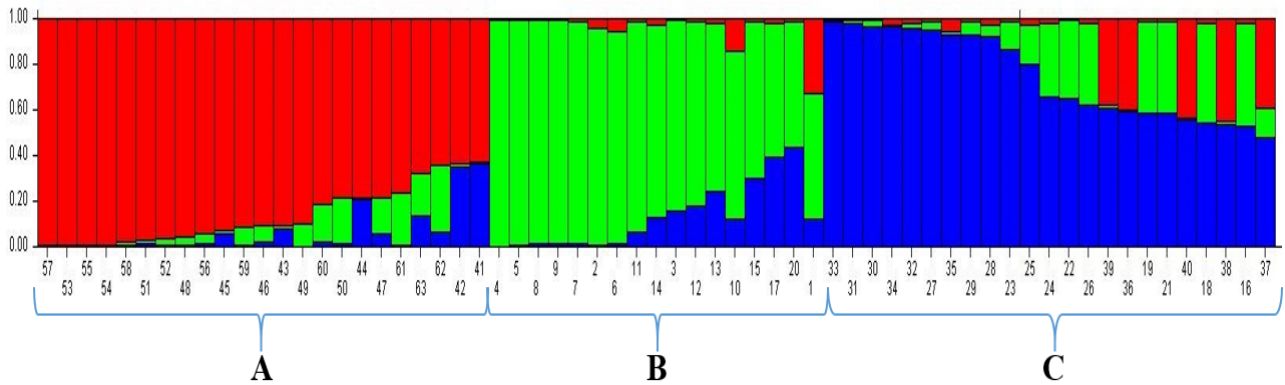
Source	Degree of Freedom (df)	Sum of Squares (SS)	% of Total Variance
Among Pops	8	4.119	1%
Within Pops	54	26.754	99%
Total	62	30.873	100%

### 3.4. Population Genetic Structure Analysis for iPBS Markers

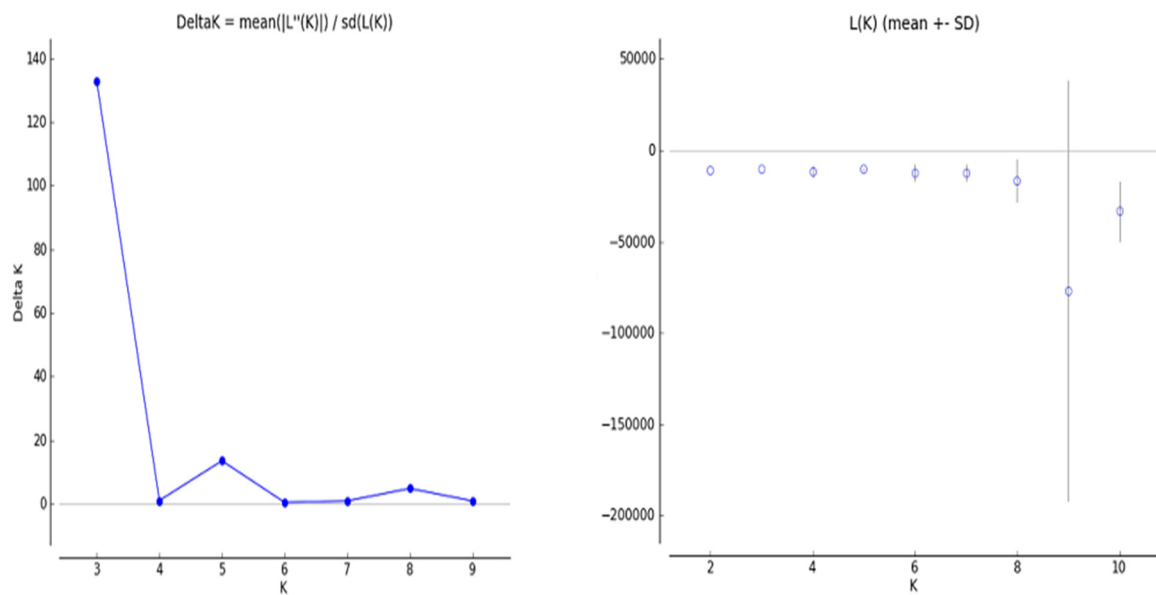
To understand the population structure among the 63 wheat varieties, we divided each entry into corresponding subgroups using the model-based approach in the STRUCTURE software. The  $\Delta K$  value is used to calculate the optimal K value. An iPBS-retrotransposon was used for the STRUCTURE analysis, where the number of groups (K) varied from 1 to 10. The estimated  $\Delta K$  value was 927.4 for the 63 wheat genotypes, which represented the three subpopulations (Figure 3). The result of genetic structure analysis suggests that the greatest value of K was calculated as 3 (Group A [red], Group B [green], and Group C [blue]) (membership probability <0.8) (Figure 4). At K = 3, group A included 15 wheat genotypes: G57, G53, G55, G54, G58, G51, G52, G48, G56, G45, G59, G46, G43, G49, and G60. Group B contained 13 wheat genotypes, including G4, G5, G8, G9, G7, G2, G6, G11, G14, G3, G12, G13, and G10. Group C included 11 genotypes, counting G33, G31, G30, G34, G32, G27, G35, G29, G28, G23, and G25. Furthermore, G50, G44, G47, G61, G63, G62,



G42, G41, G15, G17, G20, G1, G24, G22, G26, G39, G36, G19, G21, G40, G18, G38, G16, and G37 were placed in mixed groups (38.09%; membership probability <0.8). The F-statistic (Fst) value was determined as 0.4005, 0.2374, and 0.3773 in the first to third subpopulations, respectively. Likewise, the expected heterozygosity values (He) were determined as 0.2203, 0.2599, and 0.2155 in the first to third subpopulations, respectively (Tables 7 and 8).



**Figure 3.** Genetic structure of varieties according to iPBS data (the wheat varieties given in K = 3 (Figure 4) are presented in Table 7).



**Figure 4.** Line graphs from the mixture model of Ln P (D) and ΔK for wheat populations.

**Table 7.** Membership coefficients of three subpopulations of wheat varieties.

Code Number	Subpopulation			Code Number	Subpopulation		
	I	II	III		I	II	III
G1	0.327	0.546	0.127	G33	0.007	0.005	0.989
G2	0.037	0.950	0.013	G34	0.022	0.010	0.968
G3	0.006	0.831	0.163	G35	0.053	0.012	0.935
G4	0.004	0.989	0.007	G36	0.397	0.008	0.594
G5	0.007	0.979	0.014	G37	0.390	0.129	0.481
G6	0.055	0.927	0.018	G38	0.443	0.020	0.537
G7	0.011	0.973	0.016	G39	0.377	0.010	0.613
G8	0.006	0.979	0.015	G40	0.435	0.005	0.559
G9	0.006	0.976	0.017	G41	0.622	0.007	0.371

Table 7. Cont.

Code Number	Subpopulation			Code Number	Subpopulation		
	I	II	III		I	II	III
G10	0.137	0.738	0.125	G42	0.635	0.011	0.354
G11	0.008	0.922	0.070	G43	0.901	0.016	0.083
G12	0.008	0.813	0.179	G44	0.779	0.013	0.208
G13	0.015	0.740	0.245	G45	0.927	0.012	0.061
G14	0.027	0.839	0.135	G46	0.906	0.070	0.024
G15	0.009	0.689	0.302	G47	0.779	0.162	0.059
G16	0.021	0.450	0.530	G48	0.957	0.033	0.010
G17	0.016	0.588	0.396	G49	0.895	0.100	0.005
G18	0.020	0.435	0.545	G50	0.782	0.200	0.017
G19	0.011	0.400	0.589	G51	0.971	0.012	0.017
G20	0.011	0.548	0.440	G52	0.961	0.025	0.014
G21	0.014	0.398	0.588	G53	0.989	0.007	0.004
G22	0.005	0.342	0.653	G54	0.986	0.009	0.004
G23	0.012	0.117	0.871	G55	0.988	0.007	0.006
G24	0.021	0.315	0.664	G56	0.936	0.049	0.014
G25	0.023	0.175	0.802	G57	0.991	0.005	0.004
G26	0.020	0.352	0.628	G58	0.974	0.017	0.009
G27	0.009	0.036	0.955	G59	0.909	0.081	0.010
G28	0.024	0.049	0.927	G60	0.811	0.162	0.027
G29	0.009	0.061	0.930	G61	0.763	0.228	0.008
G30	0.006	0.026	0.968	G62	0.642	0.291	0.067
G31	0.005	0.010	0.986	G63	0.674	0.187	0.140
G32	0.018	0.021	0.961				

Table 8. Expected heterozygosity (He) and Fst values in three wheat subpopulations.

Subpopulation (K)	Expected Heterozygosity (He)	Fst
1	0.2203	0.4005
2	0.2599	0.2374
3	0.2155	0.3773
Mean	0.2319	0.3384

#### 4. Discussion

The determination of genetic variation and population structure in plants is important for the future and progress of plant breeding programs [50]. A rational study has been documented for the characterization of the bread wheat genotype and its wild relatives using several types of molecular markers [51]. Molecular markers such as iPBS play a critical role in the selection of targeted parents, independent of external ecological factors, to reveal the genetic relationships among plant species. Along with the role of retrotransposons in the diversification of genetic material, it is stated that retrotransposon activation is one of the key factors involved in host adaptation to ecological differences [52]. The iPBS markers used in our study helped identify wheat (*Triticum aestivum* L.) accessions at the molecular level. The obtained data provided important information about the genetic relationships among wheat accessions. The information obtained by the iPBS marker system indicates that it can be usefully used for diversity studies and genetic analysis of wheat accessions. Similarly, Nadeem [16] used the iPBS marker system for the molecular characterization of bread wheat landraces and cultivars. In addition, Demirel, [53] used iPBS-retrotransposon markers for the molecular characterization of emmer and durum wheat.

Total and polymorphic bands reported in this study were higher than Nadeem [16] and Demirel [53] using iPBS markers, as well as Kumar et al. [54] using ISSR markers, Alshehri et al. [55] using SCoT and ISSR primers, and Çifçi and Yağdı [56] using RAPD markers. The PIC value is an important piece of information in scoring the efficacy of polymorphic

loci and demonstrating the discriminative power of a primer [57]. In our study, the PIC varied between 0.048 (iPBS-2087) and 0.279 (iPBS 2380 and iPBS 2381) (mean 0.175). In a similar study of wheat in which iPBS markers were used, PIC values were reported between 0.12 and 0.25 (mean 0.19) [53]. The results differ from those of [58], who found PIC values between 0.306 and 0.913 (mean 0.660) in their study with iPBS-retrotransposons in wheat. In addition, PIC values ranged from 0.702 to 0.11, and the mean PIC value was 0.42 via Nadeem [16], who used the iPBS marker for the molecular characterization of 74 bread wheat accessions, including a total of 54 landraces and 20 commercial cultivars. This suggests that the difference in results between studies is probably due to the different genotypes and the use of fewer markers by other researchers.

The mean number of effective alleles ( $n_e$ ), genetic diversity of Nei ( $h$ ), and Shannon's information index ( $I$ ) value of the wheat varieties were calculated as 1.157, 0.95, and 0.144, respectively. In a similar study conducted in wheat, the mean  $n_e$ ,  $h$ , and  $I$  values were 1.950, 0.489, and 0.502, respectively [58]. These values were higher than in our study. A higher number of effective alleles is an indication of higher genetic variation and is therefore always desirable. In a study conducted by Kumar et al. [54] and Nadeem [16], higher gene diversity was observed for the primers producing higher alleles. In addition, Shannon's information index is one of the important criteria that distinguishes genetic variation within a population and makes variation more meaningful. Shannon's mean information index observed in the present report was lower than Nadeem's [16] and Demirel's [53]. The presence of lower values for various diversity indices in this study might be due to differences in germplasm. The Nei's genetic distance revealed G1 (Aksel 200) and G63 (Karasu 90) as genetically distinct accessions. Therefore, Aksel and Karasu accessions can be used for future bread wheat breeding. Arystanbekkyzy et al. [58] stated that genetically distinct accessions can be helpful to start breeding activities for favorable traits.

The UPGMA dendrogram placed the 63 wheat genotypes into three clusters. Clusters I, II, and III included 14 (22.25%), 14 (22.25%), and 35 (55.5%) genotypes, respectively. The UPGMA analysis showed admixture of accessions because accessions from various provinces were grouped under the same sub-groups. This result was similar to Nadeem [16]. Uncovering genetic variations occurring between populations of plant species and the information obtained are valuable for breeding and conservation of genetic resources [59]. The population structure within each wheat genotype or cultivar can be used to make crossbreeding studies more efficient. There was concordance between the population structure identified in this study and distance-based clustering from principal coordinate analysis (PCoA). In our study, it can be thought that intraspecies crossings may produce stronger hybrids due to greater genetic distances. During this study, winter and alternative bread wheat genotypes were used as plant material. Therefore, calculated diversity indices among genotypes showed the presence of fewer genetic variations. Our result is in contrast with Nadeem [16] and Demirel [53]. This can be due to the use of different genetic materials. In addition, Keser et al. [6] evaluated 22 registered winter bread wheat varieties developed for rainfed conditions under dryland conditions and presented the pedigree information of the varieties, of which 17 genotypes were common with our study. Based on pedigree information of varieties, cv. Sonmez-2001 was originated from cv. Bezostaya-1. In our study, cv. Sonmez-2001 fell into a different PCoA group than cv. Bezostaya-1. There could be two explanations for this phenomenon. One could be due to the limitations of iPBS markers. There could be similar mobility or a similar pattern of retrotransposon elements in these cultivars. Therefore, iPBS markers could not sufficiently differentiate between these two genotypes or identify genetic differences as these genotypes are in the genic region of the genome. A second explanation would be that iPBS markers work well. These two genotypes could be different genotypically. As it was seen in the pedigree of cv. Sonmez-2001, we see the contribution of other four genotypes (Tevere/3/Kremena/Lovrin-29/4/Katya-1) into the Sonmez-2001 genotypic background. Indeed, cv. Sonmez-2001 has been genetically separated from Bezostaya in every generation.

During this study, bread wheat cultivars were used as plant material. Therefore, calculated diversity indices among cultivars showed the presence of higher genetic variations among cultivars. PCoA analysis was also performed to reveal genetic relationships among wheat inclusions, and in the first three axes, PCoA analysis explained 60.10% of the total variation. As a result of the PCoA analysis, it is stated that the data obtained from this matrix is generally safe if the axes explain 25% or more of the total variation [60]. The PCoA analysis supported the clustering of the model-based structure algorithm and separated the Turkish bread wheat germplasm into three populations.

The AMOVA results revealed that the maximum genetic variations in Turkish bread wheat germplasm are present within the population. The result from the molecular analysis of variance (99%) revealed high variation among wheat accessions. Many factors, such as pollination patterns of species, selection, gene flow between locations, and variation in genotypes, affect the variation [41]. Thus, it is stated that Turkish bread wheat germplasm has a great level of genetic variation within the population that can be helpful for the breeding of this crop in the future. The findings revealed that wheat genotypes were divided into three groups according to genetic structure analysis. The results of the AMOVA were supported by previous studies as they also revealed higher genetic variations within populations [16,61].

The structure algorithm has been proven more trustworthy and informative compared to other clustering algorithms [62]. Recently, it has become important to understand population structure, which allows for the selection of various parents for use in breeding programs and the mapping of marker-trait relationships. Analysis of population structure is an important tool for estimating the levels of similarity between individuals and subpopulations, or, in other words, for understanding population structure. The model-based structure algorithm grouped 63 bread wheat accessions into three populations on the basis of their collection points. Population A was found to be larger than population B and C by accounting for 23.80% (15 genotypes) of its accessions. Population B accounted for a total of 13 accessions (20.63%), and population C was found to be the lowest. The other two populations accounted for a total of 11 accessions (17.46%), as well as mixed groups with 38.09% and a total of 24 accessions (membership probability <0.8). Cömertpay et al. [35] showed that in the STRUCTURE analysis, the number of groups (K) varied from 1 to 10. The estimated  $\Delta K$  value was 302.29 for the 81 rice accessions, which represented the three subpopulations. The population structure analysis revealed that all of the varieties used in this study were derived from three subpopulations, or three gene pools. Throughout the evolutionary process, varieties accumulate several living mutations that form the basis of genetic diversity. Moreover, forces such as recombination, random drift, and natural selection shape the genetic makeup of populations. The results show that mixed groups have the highest amount, an indication that genotypes have crossing with each other over the years and how narrow range of the genetic pool.

## 5. Conclusions

Determining and revealing genetic diversity for plant breeding is extremely important, and there are many tools and methods to reveal this diversity. However, it is very important information to reveal the genetic distance of genotypes or accessions that have not been adequately defined or are not yet known in plant breeding programs. Although classical breeding studies have been successfully applied to many plant species and cultivars, molecular markers provide invaluable information for shortening the breeding period and for more reliable cultivar development studies. In addition, the determination of genetic distance between cultivars by molecular markers makes an important contribution to the creation of new populations and the emergence of heterosis and high-yielding cultivars. Evaluation of genetic variation among wheat genotypes is important for breeding programs and the sustainability of genetic diversity, as well as for the conservation and breeding of existing genetic material. Genetic diversity studies with the use of molecular markers provide essential and critical information about population structure. Molecular

markers with high informative power, such as iPBS, have been preferred in the research of wheat population structure and genetic diversity in recent years, and their strength cannot be ignored. In conclusion, in this study, we tried to generate pre-breeding data for the selection of suitable parents that can be used in breeding studies via the iPBS-retrotransposon marker system. According to the obtained data, we demonstrated that the iPBS marker system is a method that provides easy and important information to determine the variation between wheat accessions. Population A from structure clustering was found to be more diverse, and accessions belonging to this population should be considered for future wheat breeding. According to the information obtained in the study, the most genetically distant genotypes were G1 (Aksel 2000) and G63 (Karasu 90) accessions, which can be used for future bread wheat breeding. It is thought that in future breeding programs, the agronomic and morphological characteristics of these genotypes can be evaluated according to their responses to stress factors. The data obtained reveals the diversity in wheat genotypes/varieties collected from different regions of Turkey and will provide integrity in wheat identification studies while forming the basis for wheat breeding/crossbreeding programs to be implemented in the coming years.

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