agronomy

Article

Revealing Genetic Diversity and Population Structure in Türkiye's Wheat Germplasm Using iPBS-Retrotransposon Markers

Fatih Demirel [1,*], Bünyamin Yıldırım [2], Barış Eren [1], Serap Demirel [3], Aras Türkoğlu [4], Kamil Haliloğlu [5], Kamila Nowosad [6,*], Henryk Bujak [6,7] and Jan Bocianowski [8]

1 Department of Agricultural Biotechnology, Faculty of Agriculture, Igdır University, Igdir 76000, Türkiye; baris.eren@igdir.edu.tr
2 Department of Field Crops, Faculty of Agriculture, Igdır University, Igdir 76000, Türkiye; bunyamin.yildirim@igdir.edu.tr
3 Department of Molecular Biology and Genetics, Faculty of Science, Van Yüzüncü Yıl University, Van 65080, Türkiye; serapdemirel@yyu.edu.tr
4 Department of Field Crops, Faculty of Agriculture, Necmettin Erbakan University, Konya 42310, Türkiye; aras.turkoglu@erbakan.edu.tr
5 Department of Field Crops, Faculty of Agriculture, Ataturk University, Erzurum 25240, Türkiye; kamilh@atauni.edu.tr
6 Department of Genetics, Plant Breeding and Seed Production, Wrocław University of Environmental and Life Sciences, Grunwaldzki 24A, 53-363 Wrocław, Poland; h.bujak@coboru.gov.pl
7 Research Center for Cultivar Testing, Słupia Wielka 34, 63-022 Słupia Wielka, Poland
8 Department of Mathematical and Statistical Methods, Poznań University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland; jan.bocianowski@up.poznan.pl
* Correspondence: fatih.demirel@igdir.edu.tr (F.D.); kamila.nowosad@upwr.edu.pl (K.N.)

Abstract: Investigating the genetic diversity and population structure of wheat germplasm is crucial for understanding the underlying variability essential for breeding programs and germplasm preservation. This research aims to contribute novel insights with respect to the genetic makeup and relationships among these wheat genotypes, shedding light on the diversity present within the Turkish wheat germplasm. In this study, iPBS-retrotransposon markers were employed to analyze 58 wheat genotypes, encompassing 54 landraces and 4 cultivars sourced from Türkiye. These markers serve as genetic indicators that can be used to evaluate genetic variation, build genealogical trees, and comprehend evolutionary connections. The PCR products were visualized on agarose gel, and bands were scored as present/absent. The ten iPBS primers collectively yielded an average of 16.3 alleles, generating a total of 163 polymorphic bands. The number of alleles produced by individual markers ranged from 4 (iPBS-2386) to 29 (iPBS-2219). The genetic parameters were calculated using the popgen and powermarker programs. The genetic relationships and population structures were assessed using the ntsys and structure programs. Polymorphism information content (PIC) per marker varied from 0.13 (iPBS-2390) to 0.29 (iPBS-2386), with an average value of 0.22. Shannon's information index (I) was calculated as 1.48, while the number of effective alleles (Ne) and Nei's genetic diversity (H) were determined to be 0.26 and 0.31, respectively. Genotype numbers 3 (*Triticum dicoccum*) and 10 (*Triticum monococcum*) exhibited the maximum genetic distance of 0.1292, signifying the highest genetic disparity. Population structure analysis revealed the segregation of genotypes into three distinct subpopulations. Notably, a substantial portion of genotypes clustered within populations correlated with the wheat species. This population structure result was consistent with the categorization of genotypes based on wheat species. The comprehensive assessment revealed noteworthy insights with respect to allele distribution, polymorphism content, and population differentiation, offering valuable implications for wheat breeding strategies and germplasm conservation efforts. In addition, the iPBS markers and wheat genotypes employed in this study hold significant potential for applications in wheat breeding research and germplasm preservation.

Citation: Demirel, F.; Yıldırım, B.; Eren, B.; Demirel, S.; Türkoğlu, A.; Haliloğlu, K.; Nowosad, K.; Bujak, H.; Bocianowski, J. Revealing Genetic Diversity and Population Structure in Türkiye's Wheat Germplasm Using iPBS-Retrotransposon Markers. *Agronomy* **2024**, *14*, 300. https://doi.org/10.3390/agronomy14020300

Academic Editor: Enrico Porceddu

Received: 5 December 2023
Revised: 11 January 2024
Accepted: 24 January 2024
Published: 30 January 2024

Keywords: interprimer binding site; germplasm; genetic diversity; *Tiriticum monococcum*; *Triticum dicoccum*; wheat

1. Introduction

Wheat stands prominently as a fundamental dietary staple in global human nutrition, recognized for its indispensability [1]. The success of this agricultural commodity hinges on its adaptability and potential for high yields. Notably, the presence of the gluten protein fraction assumes a pivotal role, conferring the viscoelastic properties essential for the processing of dough into diverse food products such as bread, pasta, and noodles, among others [2]. Beyond its culinary significance, wheat enriches the human diet by providing essential amino acids, minerals, vitamins, phytochemicals, and dietary fiber components. These nutritive elements are particularly abundant in whole-grain products [3].

The cultivated species of wheat, scientifically classified as *Triticum* L., are systematically grouped into three distinct subcategories based on their chromosomal composition: (i) diploid einkorn (*Triticum monococcum* ssp. *monococcum*) (2n = 14, AA), (ii) tetraploid emmer (*Triticum dicoccum* L.) and durum wheat (*Triticum durum* Desf.) (2n = 28, AABB), and (iii) hexaploid spelt (*Triticum spelta* L.) and bread wheat (*Triticum aestivum* L.) (2n = 42, AABBDD) [4].

The Fertile Crescent is widely acknowledged as the presumed center of origin and diversity for wheat [5]. In terms of wheat domestication, pivotal locations include the southeastern region of Türkiye and northern Syria [6]. Türkiye's wheat diversity assumes a critical global role, providing valuable genetic resources for wheat enhancement. The emergence of durum wheat, characterized by easy husk separation, can be traced back to the eastern Mediterranean region [7]. Through the analysis of restriction fragment length polymorphism data (RFLP), it has been discerned that durum wheat has superseded its precursor, *T. dicoccum*, becoming the predominant cultivated variety of allotetraploid wheat [8]. Despite the broader cultivation of bread and durum wheats, the cultivation of einkorn and emmer wheat species persists, thanks to the efforts of smaller farmers [9].

The examination of genetic diversity in plants assumes a pivotal role in plant genetics, breeding, conservation, and evolution [10]. To effectively leverage existing gene resources in wheat breeding, a comprehensive understanding of these resources' properties, coupled with targeted breeding experiments, is essential [11]. Wheat's genetic diversity, akin to other crops, has diminished due to domestication and rigorous selection methods in contemporary plant breeding programs [12]. Consequently, there has been a reduction in on-farm genetic diversity and a decline in regionally well-adapted and genetically distinct landraces.

The genes that provide resistance to diseases, pests, and environmental pressures may be found in many genetic sources, even when these genes are not present in the crop types that are now in use [13]. These valuable genes may be present in wild species and traditional landraces [14,15]. The remaining portions of these gene pools are commonly referred to as genetic resources, as emphasized in the current literature [11]. Plant breeders rely heavily on these genetic resources to cultivate novel agricultural plant varieties. The essential process of germplasm characterization is integral to breeding endeavors, enabling breeders to identify unique genetic variants for use in marker-assisted breeding [16]. Molecular markers have become indispensable tools for unraveling the genetic diversity of wheat [12,17]. They have significantly transformed breeding research, streamlining the time required to complete breeding studies [18]. Notably, molecular markers remain unaffected by environmental factors, allowing for a more precise estimation of genetic variation at the DNA level [19].

Continuous progress in scientific research has led to the development of molecular markers with diverse qualities [20]. Various molecular marker techniques have been employed to analyze genetic diversity and associations among different Triticum species.

These methods encompass amplified fragment length polymorphism (AFLP) [21], inter-simple-sequence repeat (ISSR) [22], simple-sequence repeat (SSR) [23], diversity array technology (DArT) [12], random amplified polymorphic DNA (RAPD) [24], start codon targeted (SCoT) markers [25], expressed sequence tag (EST) [26], single-nucleotide polymorphism (SNP) [27], and next-generation sequencing (NGS) [28]. Each of these molecular marker techniques offers distinct advantages and contributes to a comprehensive understanding of the genetic landscape of wheat. Within the array of molecular markers, retrotransposons stand out as genetic elements capable of prolific reproduction and mobility, comprising substantial portions of the genomes across various eukaryotic organisms [29]. Retrotransposons (RTNs) play a crucial role in fostering genetic diversity in plants. Notably, in numerous plant species characterized by expansive genomes, retrotransposons constitute over 50% of the nuclear DNA [30]. The concept of utilizing iPBS-retrotransposons as a comprehensive marker applicable to both animal and plant species was introduced by Kalendar et al. [31]. iPBS-retrotransposons have since been employed in molecular characterization, phylogenetic analysis, and evolutionary research across a variety of crop plants [15].

The iBPS-retrotransposon marker has been employed in wheat [18,29]. However, there has been a limited exploration such as with respect to the type of germplasm or the characterization of wheat populations using iBPS-retrotransposons. Consequently, the current study focuses on genetically characterizing and elucidating the population structure of Turkish wheat genotypes, encompassing *T. durum*, *T. dicoccum*, *T. monococcum*, and *T. aestivum*, with the primary objective of evaluating genetic diversity. Therefore, the purpose of this study was to characterize Türkiye wheat germplasm with different ploidy levels for the purpose of assessing genetic diversity and investigating the population structure.

2. Materials and Methods

2.1. Plant Materials

The study utilized a total of 58 genotypes, categorized as follows: 13 durum wheat genotypes (*T. durum*), 20 bread wheat genotypes (*T. aestivum*), 16 emmer wheat genotypes (*T. dicoccum*), 5 einkorn wheat genotypes (*T. monococcum*), and 4 cultivars (Ahmetağa, Aydın-93, Fırat-93, and Cemre) as plant material (Figure 1, Table 1, Supplementary Table S1). These wheat genotypes are now accessible at the department of agriculture at Iğdır University in Türkiye.

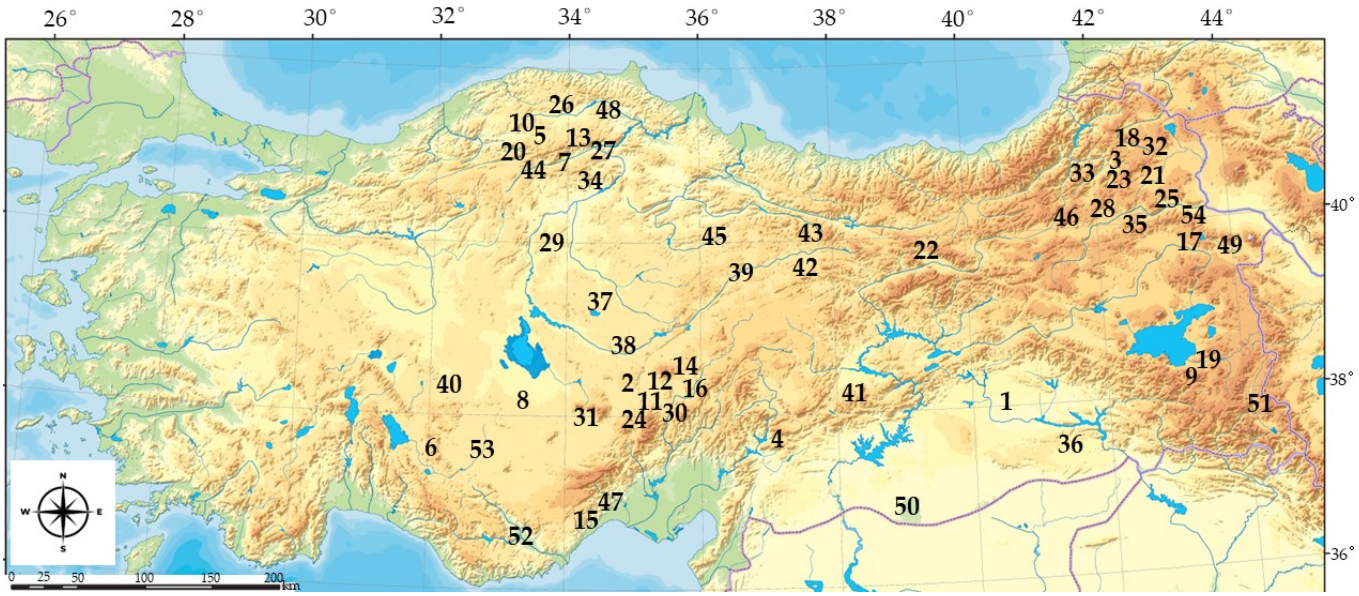

Figure 1. Geographical images of the locations where the wheat genotypes used in the study were collected.

Table 1. Details of the wheat germplasm collection.

No.	Province	Town	Species	No.	Province	Town	Species
1	Diyarbakır	Center	*T. durum*	30	Kayseri	Epçe	*T. aestivum*
2	Kayseri	Epçe	*T. dicoccum*	31	Niğde	Center	*T. aestivum*
3	Kars	Kuyucuk	*T. dicoccum*	32	Kars	Geçit	*T. durum*
4	Kahramanmaraş	Elbistan	*T. aestivum*	33	Kars	Küçükçatma	*T. dicoccum*
5	Kastamonu	İhsangazi	*T. monococcum*	34	Kastamonu	İhsangazi	*T. monococcum*
6	Konya	Center	*T. aestivum*	35	Kars	Geçit	*T. durum*
7	Kastamonu	İhsangazi	*T. dicoccum*	36	Mardin	Center	*T. aestivum*
8	Aksaray	Center	*T. aestivum*	37	Yozgat	Center	*T. aestivum*
9	Van	Gedelova	*T. aestivum*	38	Kayseri	Yemliha	*T. aestivum*
10	Kastamonu	İhsangazi	*T. monococcum*	39	Sivas	Divriği	*T. aestivum*
11	Kayseri	Yeniköy	*T. dicoccum*	40	Konya	Merkez	*T. aestivum*
12	Kayseri	Pınarbaşı	*T. aestivum*	41	Adıyaman	Merkez	*T. aestivum*
13	Kastamonu	İhsangazi	*T. dicoccum*	42	Sivas	Merkez	*T. aestivum*
14	Kayseri	Hoşça	*T. dicoccum*	43	Sivas	Gemerek	*T. durum*
15	Mersin	Silifke	*T. durum*	44	Kastamonu	İhsangazi	*T. dicoccum*
16	Kayseri	Develi	*T. dicoccum*	45	Sivas	Gürün	*T. aestivum*
17	Iğdır	Center	*T. durum*	46	Kars	Güvercin	*T. dicoccum*
18	Kars	Geçit	*T. aestivum*	47	Mersin	Merkez	*T. durum*
19	Van	Center	*T. aestivum*	48	Kastamonu	İhsangazi	*T. monococcum*
20	Kastamonu	İhsangazi	*T. dicoccum*	49	Iğdır	Center	*T. durum*
21	Kars	Center	*T. dicoccum*	50	Şanlıurfa	Center	*T. durum*
22	Erzincan	Center	*T. aestivum*	51	Van	Gedelova	*T. aestivum*
23	Kars	Büyükçatma	*T. dicoccum*	52	Mersin	Çarkçılı	*T. durum*
24	Kayseri	Gümüşören	*T. aestivum*	53	Konya	Merkez	*T. durum*
25	Kars	Center	*T. dicoccum*	54	Kars	Duraklı	*T. dicoccum*
26	Kastamonu	Center	*T. durum*	55	Ahmetağa	Bahri Dagdas IARI [1]	*T. aestivum*
27	Kastamonu	İhsangazi	*T. monococcum*	56	Aydın-93	GAP IARTC [2]	*T. durum*
28	Kars	Büyükçatma	*T. dicoccum*	57	Fırat-93	GAP IARTC	*T. durum*
29	Çankırı	Center	*T. durum*	58	Cemre	GAP IARTC	*T. aestivum*

[1] IARI, International Agricultural Research Institute; [2] IARTC, International Agricultural Research and Training Center.

2.2. DNA Extraction and PCR Amplification of Wheat Genotypes

A total of 25 seeds of each wheat accession were germinated in trays in a greenhouse. To harvest DNA from each wheat plant, the young leaves of each plant were powdered thoroughly in liquid nitrogen. The DNA extraction process was evaluated on bulked samples consisting of 10 individuals chosen at random from each accession. By utilizing the DNA extraction method proposed by Zeinalzadehtabrizi et al. [32], gDNA was extracted from 58 different genotypes. A NanoDrop ND-1000 UV/Vi spectrophotometer (Thermo Fisher Scientific Company, Waltham, MA, USA) was utilized to ascertain the concentrations of DNA contained within the sample. The iPBS primers produced by Kalendar et al. [31] were used as markers in the molecular characterization. In this investigation, a total of ten iPBS markers were chosen based on their ability to create distinct and measurable bands for all genotypes. Polymerase chain reaction (PCR) was conducted in a thermal cycler device (SensoQuest Labcycler, Göttingen, Germany) with a total capacity of 10 µL of reaction mixture.

In the reaction mixture, components comprising 3 µL of DNA (about 20 ng μL^{-1}), 0.3 µL of Taq DNA polymerase (5 U μL^{-1}), 0.5 µL of dNTP (2 mM), 1 µL of primer (20 pmol), 1 µL of $MgCl_2$ (2 mM), 4.2 µL of dH_2O, and 1 µL of 10X PCR buffer were used. The thermal profile cycle of the polymerase chain reaction (PCR) included one cycle of pre-denaturation at 94 °C for thirty seconds, forty-two cycles of amplification (94 °C for twenty-five seconds, annealing temperature (Table 2) for forty-five seconds, and 72 °C for one minute), and one cycle of final extension at 72 °C for five minutes. The PCR products were resolved on a 3% agarose gel in 1 × TBE buffer at a voltage of 120 V for a duration of 4 h. Ultimately, bands on the gel were visualized using ultraviolet (UV) light and captured as photographs by an Imager Gel Doc XR + system (Bio-Rad, Hercules, CA, USA) (Figure 2). The GeneRuler 100 bp DNA Ladder manufactured by Thermo Scientific (Emeryville, CA, USA) was used as a molecular size marker.

Table 2. Sequences and annealing temperatures of 10 iPBS-retrotransposon primers used to study genetic diversity in 58 wheat genotypes.

Number	Marker Name	Primer Sequences (5′ → 3′)	Annealing Temperature (°C)
1	IPBS-2219	GAACTTATGCCGATACCA	57
2	IPBS-2270	ACCTGGCGTGCCA	60
3	IPBS-2271	GGCTCGGATGCCA	57.5
4	IPBS-2278	GCTCATGATACCA	44
5	IPBS-2375	TCGCATCAACCA	44
6	IPBS-2377	ACGAAGGGACCA	44
7	IPBS-2378	GGTCCTCATCCA	44
8	IPBS-2383	GCATGGCCTCCA	48
9	IPBS-2386	CTGATCAACCCA	48
10	IPBS-2390	GCAACAACCCCA	44

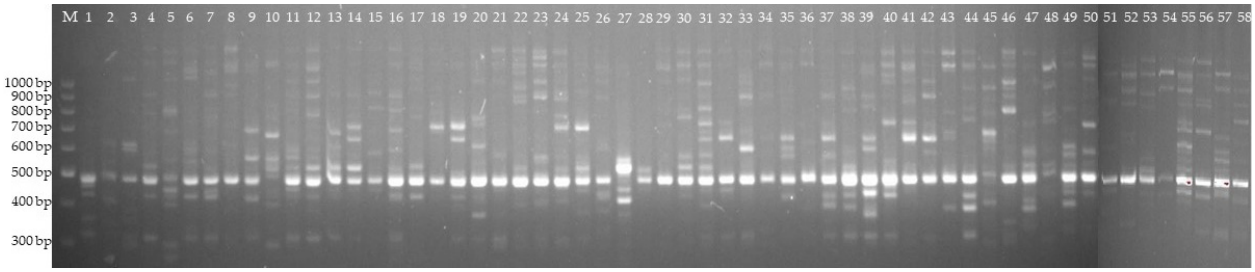

Figure 2. PCR amplification profile of wheat genotypes captured using the iPBS-2219 marker. M, 100-base-pair DNA ladder.

2.3. iPBS Data Scoring and Analysis

In the scoring process, only robust and distinct bands were taken into consideration. The band pictures that were acquired via the use of iPBS-retrotransposons were scored as binary data, and the results were entered using Excel software. A value of 0 denotes the absence of a band, while a value of 1 represents the presence of a band. Scoring was performed with reference to a 100 bp+ DNA ladder, as illustrated in Figure 2. Using PopGene version 1.31 [33], the effective number of alleles (Ne) and Shannon's information index (I) were evaluated for each iPBS marker. Nei genetic diversity (H) and polymorphism information content (PIC) were determined using PowerMarker V3.025 software [34]. NTSYS-PC software version 2.02 was used to compute the Dice similarity index, to generate a UPGMA (unweighted pair group method with arithmetic mean) dendrogram, and to create a two-dimensional graph with PCoA (Principal coordinate analysis) [35]. Furthermore, this software was used to perform the Mantel test [36], which calculates the correlation coefficient (r) between the cophenetic values and the Dice similarity index. Using the COPH module, the cophenetic value matrix was initially computed by using the tree matrix that was obtained from the iPBS analysis. The MXCOMP module was used to investigate the goodness-of-fit value (r) representing the relationship between the UPGMA dendrogram and the Dice similarity index matrix [37]. To undertake an analysis of the population structure of wheat varieties, STRUCTURE version 2.3.4 was utilized [38]. The appropriate number of subpopulations (delta K value) was determined using the approach described by Evanno et al. [39] using the STRUCTURE HARVESTER program [40]. The STRUCTURE program was used to determine the expected heterozygosity (He) and fixation index (F_{ST}) values that reflect populations [37].

3. Results

3.1. Polymorphism Disclosed by iPBS Primer

Distinct and scorable bands were successfully obtained from each primer utilized in the investigation, as outlined in Table 3. In total, 168 bands were generated across the 20 employed primers. Among these, 163 bands were both visible and quantifiable,

representing polymorphic variations. The allelic diversity observed among the primers ranged from 4 alleles (iPBS 2386) to 29 alleles (iPBS 2219), with an average of 16.3 alleles. iPBS marker analysis unveiled a spectrum of polymorphic information content (PIC) values, with iPBS 2390 exhibiting the lowest value of 0.13 and iPBS 2386 displaying the highest value, at 0.29. The mean PIC value across all markers was computed as 0.22. The polymorphism rate displayed variability, ranging from 80% (iPBS 2386) to 100% (iPBS 2219, iPBS 2278, iPBS 2377, iPBS 2378, and iPBS 2383). Shannon's information index (I) revealed diverse values, ranging from the lowest value of 0.11 for iPBS 2390 to the highest value of 0.48 for iPBS 2386. The mean value of Shannon's information index across all observations was calculated to be 0.31. The effective number of alleles (Ne) ranged from 1.35 (iPBS 2390) to 1.74 (iPBS 2386), with a mean value of 1.48. These results collectively provide a comprehensive overview of the genetic diversity and informative parameters derived from the iPBS marker analysis conducted in the investigated wheat germplasm.

Table 3. Genetic parameters of iPBS markers used for 58 genotypes.

Marker Name	TNB [1]	NPB	PR (%)	H	PIC	I	Ne
IPBS-2219	29	29	100	0.23	0.20	0.29	1.39
IPBS-2270	19	18	94.7	0.28	0.23	0.31	1.61
IPBS-2271	21	20	95.2	0.25	0.21	0.38	1.40
IPBS-2278	18	18	100	0.30	0.24	0.32	1.54
IPBS-2375	10	9	90	0.23	0.19	0.24	1.38
IPBS-2377	19	19	100	0.31	0.25	0.42	1.52
IPBS-2378	20	20	100	0.24	0.20	0.18	1.41
IPBS-2383	16	16	100	0.28	0.23	0.34	1.46
IPBS-2386	5	4	80	0.36	0.29	0.48	1.74
IPBS-2390	11	10	90.9	0.15	0.13	0.11	1.35
Total	168	163					
Mean	16.8	16.3	95.08	0.26	0.22	0.31	1.48

[1] TNB: total number of bands; NPB: number of polymorphic bands; PR: polymorphism rate; H: genetic diversity of Nei; PIC: polymorphic information content; I: Shannon's information index; Ne: effective number of alleles.

3.2. Genetic Distance and Cluster Analysis for Wheat Genotypes

The Dice method was employed to assess the similarity between the 58 genotypes, resulting in the generation of similarity coefficient values for each genotype (Supplementary Table S2). The mean Dice similarity coefficient was calculated to be 0.5948. Upon scrutinizing the genotypes using the Dice similarity coefficient, it was observed that genotypes 3 and 10 exhibited the lowest similarity, registering a coefficient value of 0.1299. In contrast, genotypes 17 and 49 were identified as the most similar, with a coefficient value of 0.8829.

To further analyze the relationships among the 58 wheat genotypes, clustering analysis was conducted using the UPGMA technique, with the Dice similarity index serving as the basis for the study. The resulting dendrogram was utilized to construct the similarity matrix. Subsequently, a Mantel test analysis was performed using the Dice similarity matrix, revealing a correlation coefficient value of r = 0.96011 for the 58 wheat genotypes. This investigation provides valuable insights into the genetic relationships and clustering patterns among the examined wheat genotypes. The dendrogram visually represents variations in similarity levels, spanning from 0.29 to 0.88 (Figure 3). Initially, the dendrogram partitioned the genotypes into two primary clusters, designated as groups S1 and S2. Within the overarching group denoted as S2, additional subsidiary groups were identified. Specifically, subgroups S3 and S4 emerged within group S2. Subgroup S4 further gave rise to groups S5 and S6, with subgroup S6 leading to subgroups S7 and S8. In a similar manner, subgroup S8 contributed to the formation of subgroups S9 and S10, and subgroup S10 resulted in subgroups S11 and S12. Notably, groups S11a and S11b were delineated beneath subgroup S11, while groups S13 and S14 formed under subgroup S12. The dendrogram analysis provided insights into the degrees of separation among major groups and subgroups, denoted as S1, S2, S3, S4, S6, S7, S8, S10, S11, S11a, S11b, S12, and

S13, with separation degrees measuring approximately 0.750, 0.430, 0.548, 0.495, 0.541, 0.600, 0.582, 0.640, 0.700, 0.773, 0.710, 0.660, and 0.710, respectively. It is noteworthy that no degree of separation was observed in subgroups S5, S9, and S14 due to the clustering of individual genotypes within these subgroups. This dendrogram analysis effectively portrays the hierarchical relationships and clustering patterns among the wheat genotypes.

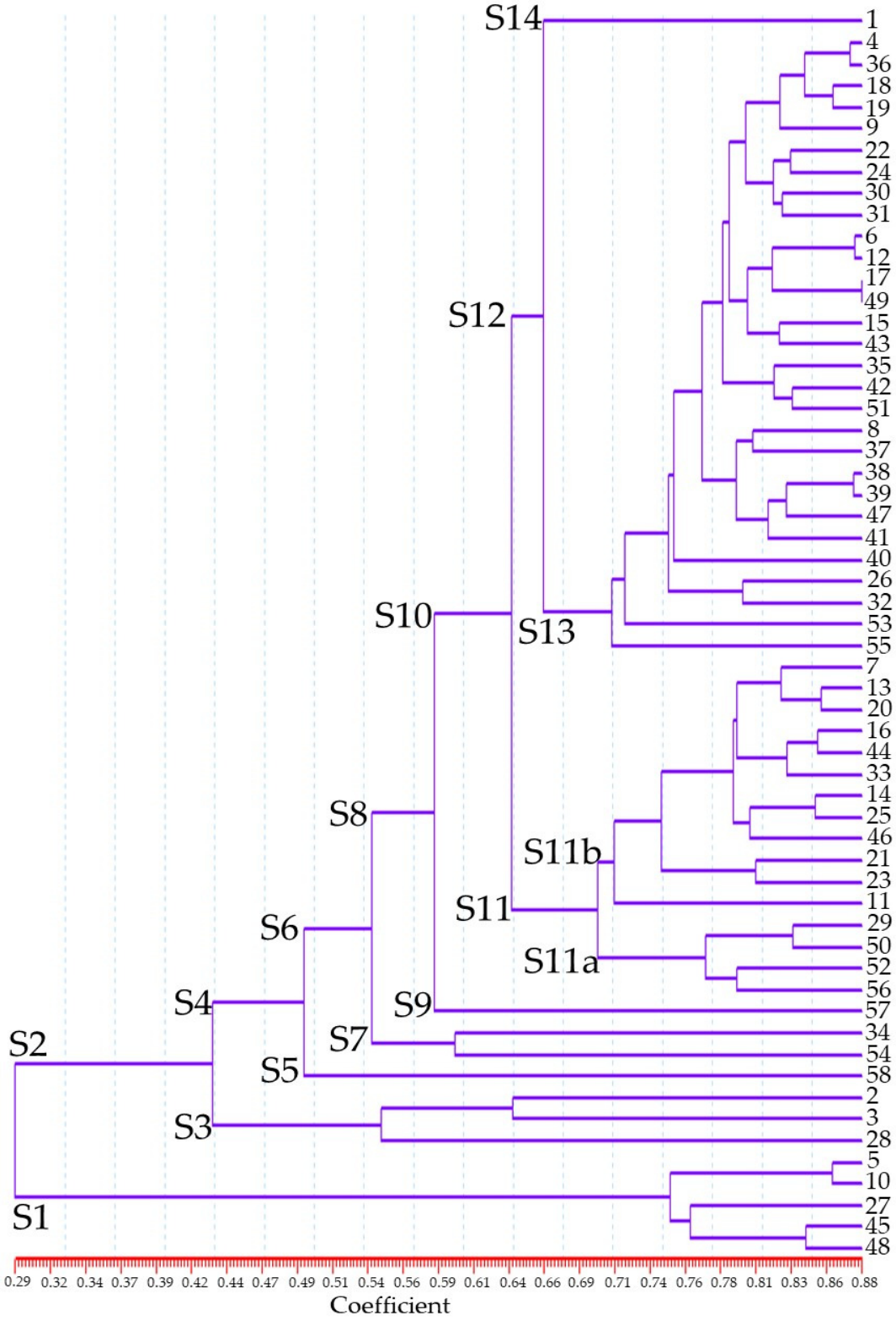

Figure 3. UPGMA dendrogram created using the Dice similarity index for 58 wheat genotypes.

Upon scrutinizing the major groups and subgroups, a distinct cluster was observed within main group S1. This cluster comprises genotypes 5, 10, 27, and 48 from *T. monococcum*, along with genotype number 45 from *T. aestivum*. Subgroup S3 exclusively includes genotypes 2, 3, and 28 from *T. dicoccum*, forming distinct clusters. Additionally, genotype number 58, specifically belonging to *T. aestivum*, exhibited a unique clustering pattern within subgroup S5. In subgroup S7, a cluster was identified where genotype 34 (*T. monococcum*) and genotype 54 (*T. dicoccum*) are grouped together. Furthermore, genotype 57 (*T. durum*) displayed a distinctive clustering pattern within subgroup S9. The study results also highlight that *T. dicoccum* formed a distinct subgroup, namely S11b, encompassing genotypes 7, 11, 13, 14, 16, 20, 21, 23, 25, 33, 44, and 46. Within subgroup S11a, it was determined that only *T. durum* exhibited clustering, with genotypes 29, 50, 52, and 56 forming a distinct subgroup. Subgroup S13, representing the *T. aestivum* species, included the following accessions: 4, 6, 8, 9, 12, 18, 19, 22, 24, 30, 31, 36, 37, 38, 39, 40, 41, 42, 51, and 55. Clustering analysis further revealed that genotypes 15, 17, 26, 32, 35, 43, 47, 49, and 53 originating from *T. durum* tended to group together. Finally, within subgroup S14, it was identified that only genotype number 1 (*T. durum*) exhibited a clustering pattern. These clustering patterns provide valuable insights into the genetic relationships and subgroupings among the examined wheat genotypes.

The similarity matrix was employed to construct a two-dimensional graph, representing the principal coordinate analysis (PCoA). The cumulative value of the first two principal coordinates accounted for 68.21% of the total variation (Figure 4). The groups that are displayed in Figure 4 and referred to as S1, S3, S5, S7, S9, S11a, S11b, S13, and S14 correspond to those presented in the separated branches in Figure 3. The results from the PCoA analysis revealed that the observed clustering patterns were consistent with the outcomes obtained from the cluster analysis (Figures 3 and 4). This alignment reinforces the robustness and reliability of the observed clustering patterns, providing a coherent representation of the genetic relationships among the wheat genotypes.

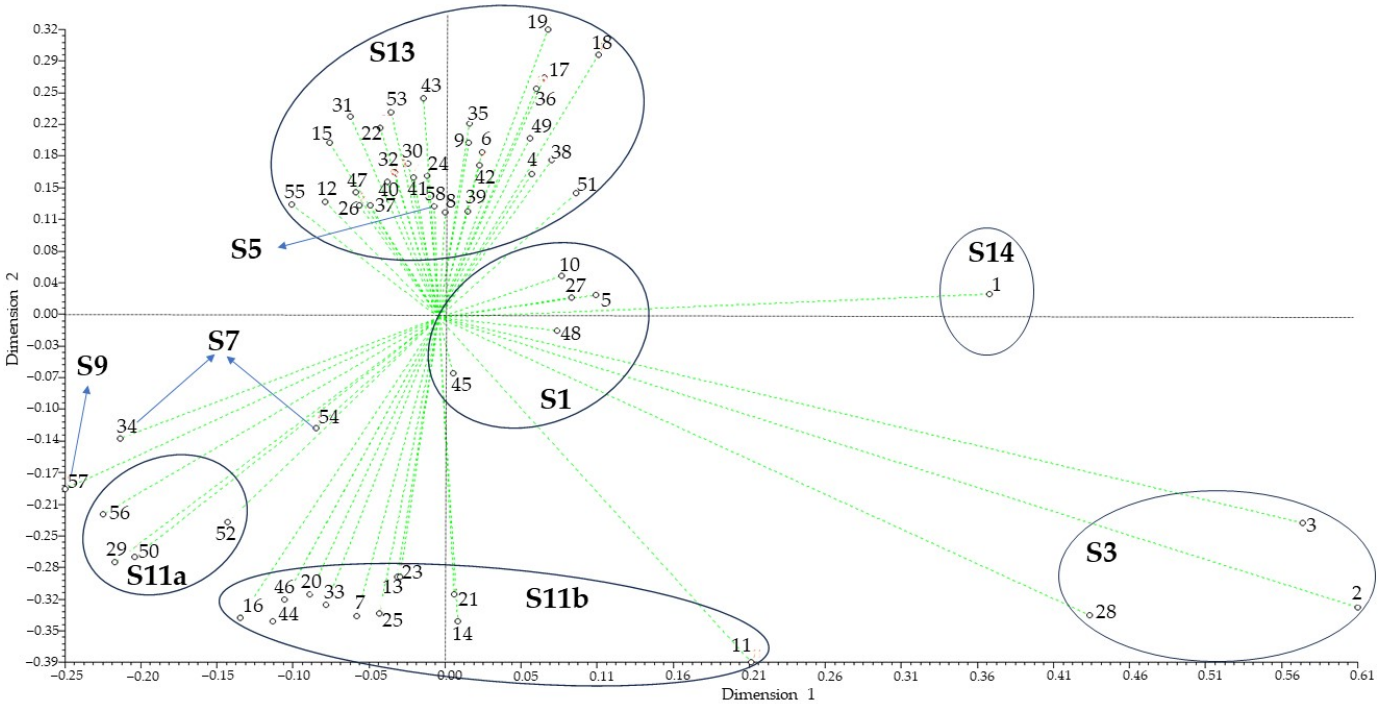

Figure 4. Two-dimensional PCoA analysis of 58 wheat accessions.

3.3. Population Genetic Structure Analysis for Wheat Genotypes

The population structure analysis for the 58 wheat genotypes involved the utilization of a model-based technique within the STRUCTURE program. To partition each entry

into respective subgroups, the Delta K values were calculated, and the STRUCTURE HARVESTER online tool was employed for this purpose (refer to Figure 5). The maximum value for Delta K was determined to be 112.629. Based on the Delta K findings, it was ascertained that the 58 wheat genotypes can be effectively classified into three distinct populations. This result provides valuable information about the underlying genetic structure within the set of wheat genotypes, facilitating a clearer understanding of the diversity and relationships among them. The STRUCTURE program's model-based approach is instrumental in delineating and characterizing subpopulations, contributing insights into the population genetics of the studied wheat germplasm.

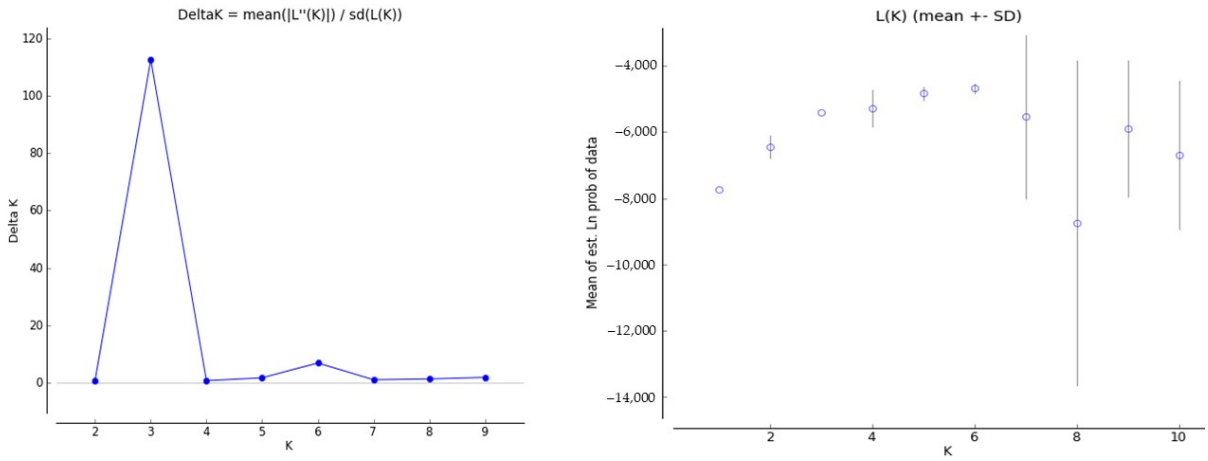

Figure 5. Line graphs from the mixture model of Ln P (D) and ΔK for the wheat population.

The population structure analysis results (Figure 6) highlight the categorization of the 58 examined genotypes into three distinct subgroups denoted as P_1, P_2, and P_3. Genotypes with a membership coefficient equal to or greater than 0.80 were considered to possess a high level of genetic purity, while five genotypes displayed hybrid characteristics. The average F_{ST} values for each subgroup were determined, quantifying population differentiation through allele correlations. Specifically, the F_{ST} values were calculated as 0.5353 for P_1 (red), 0.4416 for P_2 (green), and 0.6934 for P_3 (blue). Additionally, the analysis revealed that the P_2 population exhibited the highest expected heterozygosity value of 0.1944, while the P_1 population had the lowest expected heterozygosity value of 0.1501 (Table 4). These findings provide insights into the genetic structure and differentiation among the identified subgroups, offering a comprehensive understanding of the diversity and purity levels within the studied wheat genotypes. The utilization of membership coefficients and genetic parameters enhances the characterization of distinct populations, contributing valuable information to wheat breeding and germplasm preservation efforts.

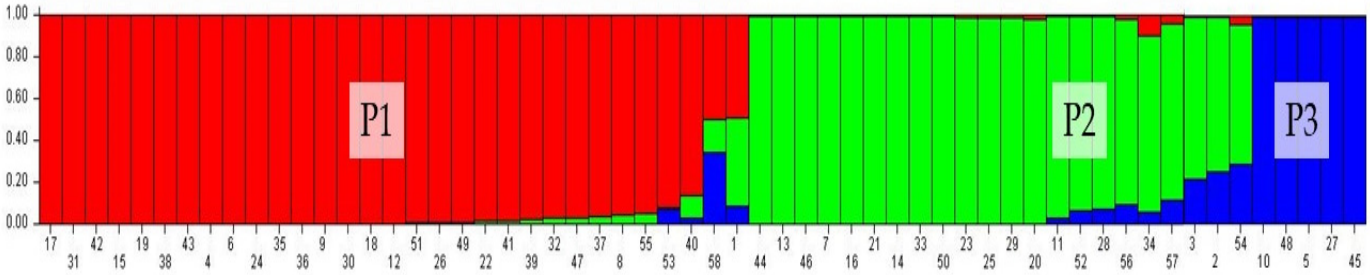

Figure 6. Result of structure analysis of the genetic population of 58 wheat genotypes (K = 3).

Table 4. Expected heterozygosity and F_{ST} (fixation index) values of subpopulations according to the results of structure analysis.

Population	Expected Heterozygosity (He)	F_{ST}
P1	0.1501	0.5353
P2	0.1944	0.4416
P3	0.1639	0.6934
Mean	0.1695	0.5567

The P1 population consists only of genotypes belonging to the bread wheat and durum wheat genotypes. The P2 population consists of several genotypes of *T. dicoccum* (genotypes 7, 11, 13, 14, 16, 20, 21, 23, 25, 28, 33, and 44) and *T. durum* (genotypes 29, 46, 50, 52, 56, and 57), together with genotype 34 (*T. monococcum*). The population denoted as P3 exhibited the presence of genotypes of *T. monococcum*, specifically designated as 5, 10, 27, and 48. Additionally, a genotype of *T. aestivum*, identified as 45, was also observed within this population (Table 5).

Table 5. Membership coefficients dividing 58 wheat genotypes into subpopulations according to the results of structure analysis.

Genotype Number	P1	P2	P3	Genotype Number	P1	P2	P3
1	0.486	0.428	0.086	30	0.996	0.004	0.001
2	0.003	0.746	0.251	31	0.999	0.000	0.000
3	0.001	0.782	0.217	32	0.969	0.028	0.003
4	0.997	0.002	0.000	33	0.005	0.995	0.000
5	0.001	0.001	0.998	34	0.093	0.848	0.059
6	0.997	0.001	0.002	35	0.997	0.003	0.001
7	0.004	0.996	0.001	36	0.997	0.002	0.001
8	0.953	0.046	0.001	37	0.964	0.033	0.003
9	0.996	0.002	0.001	38	0.998	0.002	0.000
10	0.001	0.000	0.999	39	0.978	0.021	0.001
11	0.002	0.966	0.032	40	0.863	0.102	0.035
12	0.994	0.005	0.000	41	0.980	0.018	0.002
13	0.002	0.997	0.001	42	0.999	0.001	0.000
14	0.004	0.995	0.001	43	0.998	0.001	0.002
15	0.998	0.001	0.000	44	0.001	0.999	0.001
16	0.003	0.996	0.000	45	0.001	0.000	0.998
17	0.999	0.001	0.001	46	0.002	0.997	0.001
18	0.995	0.001	0.003	47	0.968	0.032	0.001
19	0.998	0.001	0.001	48	0.001	0.000	0.999
20	0.016	0.983	0.001	49	0.990	0.009	0.000
21	0.003	0.996	0.001	50	0.005	0.994	0.000
22	0.985	0.014	0.001	51	0.992	0.002	0.006
23	0.008	0.991	0.001	52	0.003	0.927	0.071
24	0.997	0.003	0.001	53	0.920	0.002	0.079
25	0.010	0.990	0.000	54	0.037	0.676	0.287
26	0.991	0.004	0.005	55	0.949	0.048	0.003
27	0.001	0.001	0.998	56	0.017	0.884	0.099
28	0.003	0.924	0.073	57	0.037	0.845	0.119
29	0.009	0.990	0.001	58	0.496	0.159	0.345

4. Discussion

The assessment of genetic diversity and population structure in plants holds significant importance in terms of advancing plant breeding initiatives [17]. Evaluating indigenous species in regions of domestication and exploring species diversity can provide valuable insights into evolutionary patterns and the impacts of socioeconomic and geoecological

variables on genetic structure. Numerous studies have been conducted, comprehensively analyzing of wheat germplasm and its wild counterparts, and employing diverse molecular markers [6,12,17,41]. Within the realm of retrotransposon-based markers, researchers have successfully utilized the iPBS-retrotransposon marker for molecular characterization of wheat [11]. iPBS markers offer several advantages, particularly in targeting retrotransposons, which are commonly found in plant genomes [18]. The versatility of iPBS markers allows for their application across various organisms following successful implementation in one organism [31]. iPBS molecular markers exhibit high productivity due to their long primer size and strong binding affinity [42]. In this study, an average of 16.3 polymorphic bands were obtained using 10 iPBS markers. On the other hand, the mean polymorphic bands that were reported in this study were higher than those that were reported by Nazarzadeh et al. [22] using RAPD and ISSR markers, Kumar et al. [43] using ISSR markers, Alshehri et al. [44] using SCoT and ISSR primers, Çifçi and Yağdi [45] using RAPD markers, Nadeem [11] using iPBS marker, and Abbasi Holasou et al. [41] using IRAP and REMAP markers.

The polymorphic information content (PIC) value is a metric employed to gauge the effectiveness of polymorphic loci in exploring genetic diversity using markers [46]. In this investigation, the mean PIC value was determined to be 0.22. While this value is comparatively lower than those reported in other studies utilizing distinct marker systems [11,43–45,47], the mean PIC value obtained in our research exceeds the value published by Khaled et al. [48]. The observed variance in PIC values could potentially be attributed to the use of different marker systems or the presence of a variable number of genotypes and markers in the study [49]. The choice of markers, along with the specific genetic makeup of the studied wheat germplasm, can influence PIC values. Nonetheless, despite variations, the PIC value remains a valuable indicator of the informativeness of markers in capturing genetic diversity in the context of this research.

This research revealed that the average Nei's gene diversity (H) value was 0.26. Notably, the H value obtained in this study surpasses those reported in earlier wheat research utilizing iPBS markers for characterization [11]. Additionally, Shannon information index (I) value (0.31) and the effective number of alleles (Ne, 1.48) observed in this research exceed the values reported in previous studies of various plant species employing iPBS markers [11,37,50]. The heightened values observed for several diversity indices in this research may be attributed to variations in germplasm and the characteristics of the employed molecular marker [11]. The iPBS-retrotransposons marker system, known for its exceptional reproducibility and worldwide applicability, has been established in various investigations [18,19]. Consequently, this marker system can be prioritized for the molecular characterization of the wheat germplasm over other dominant marker systems [11,18]. The findings underscore the effectiveness of iPBS markers in capturing genetic diversity and providing valuable insights into the wheat germplasm under investigation.

After conducting the Mantel test, the correlation coefficient was determined to be r = 0.96011 based on the results. A correlation value of 0.9 or above indicates a strong correlation between the dendrogram and the similarity indexes, suggesting that the dendrogram accurately reflects the similarity index [51]. In a study conducted by Nasri et al. [52] on genetic diversity in bread wheat genotypes using retrotransposon-based marker systems, the cophenetic correlation coefficient was computed through the Mantel test to assess the compatibility between similarity matrices and dendrogram-derived matrices. The correlation between the cophenetic matrices derived from the IRAP and REMAP markers was found to be extremely weak (r = 0.13). Similarly, Saeidi et al. [53] examined genetic diversity and the retroelement insertion polymorphism in *Aegilops tauschii* genotypes from Iran. They computed the matrix correlation coefficient (r) to compare matrices, obtaining a result of r = 0.9297. In a study on *T. dicoccoides* genotypes, Beharav et al. [54] used the Mantel test to compare data from the RAPD marker system and three distinct genetic matrices (SSM = simple matching similarity; SD = Dice similarity; SJ = Jaccard similarity). The Mantel correlation coefficient (r) for simple matching was 0.92, whereas for Dice and

Jaccard similarities, it was 0.998 and 0.593, respectively. In the present investigation, the substantial Mantel correlation coefficient indicates the high reliability of our similarity matrix, providing strong evidence for the accuracy of the dendrogram in capturing the underlying genetic relationships among the studies wheat genotypes.

Assessing genetic diversity is a pivotal aspect of breeding research aimed at enhancing various traits in crops, including quality and yield [49]. The results of the similarity analysis and cluster analysis conducted in this investigation reveal a substantial amount of genetic diversity among all the studied genotypes. The model-based structure algorithm effectively classified the 58 wheat genotypes into three groups, primarily based on their species. However, there was not an obvious genetic discrimination of the populations according to species on UPGMA. This could be due to either an insufficient number of selected iPBS markers or an uneven distribution of the markers among different genomes of the genotypes used in this study.

It is noteworthy that only a fraction of the *T. monococcum* genome is present in hexaploid wheat. Therefore, utilizing the genetic diversity inherent in the *T. monococcum* genome can be instrumental in uncovering novel and additional traits when developing tetraploid and hexaploid cultivars [17]. A comprehensive understanding of the extent of diversity within a collection of wheat genetic resources is crucial for making significant advancements in wheat improvement. This knowledge serves as a foundation for targeted breeding efforts aimed at enhancing specific traits and overall crop performance. The conventional breeding approach relies on the identification of naturally occurring or artificially produced alleles that provide resistance to plants. These alleles are then transferred to superior genotypes using various breeding procedures. The efficacy of the conventional breeding method relies on the presence of functional diversity. Genetic diversity is constrained by the genetic bottleneck that arises during farming [55]. The introduction of diversity by natural or random stimulation is a limiting element in the breeding process and leads to unexpected outcomes in plant breeding [56]. Researchers and breeders used to look to crops' wild relatives and other plant genetic resources as a "last-option emergency solution" when contemporary elite germplasm failed to provide the desired results. Introducing desired characteristics into the cultivated background from unadopted germplasm, such as crop wild relatives or landrace materials, requires more time and money than doing so from elite lines. This is because there is low linkage drag. The advancement of technology has led to the acquisition of genome and transcriptome sequences for several plant species, marking the beginning of a new age in plant breeding [57].

5. Conclusions

Further studies on molecular diversity, agromorphological characterization, and genotype identification are imperative to safeguard the wheat germplasm and improve crops. In this study, we investigated the relatedness and diversity of wheat genotypes using iPBS-based markers to assess genetic variation across various ploidy levels of wheat. In this work, the efficiency of iPBS molecular markers in distinguishing polyploidy in wheat species was demonstrated. Despite the observed low polymorphic information content (PIC) values in the employed iPBS markers, principal coordinate analysis (PCoA), in addition to clustering and population structure analyses, revealed a significant level of genetic diversity among the wheat genotypes. Recent research on wheat population structure and genetic diversity favors molecular markers with high levels of informativeness, such as iPBS. Molecular analyses identified genotype 3 and genotype 10 as genetically distinct among the wheat genotypes, suggesting their potential as parents in future breeding experiments. Preserving these valuable genotypes by incorporating them into germplasms is crucial. This study indicates that landrace wheats (*Triticum* ssp.), known for their extensive genetic variation, hold promise as essential resources for germplasm and as valuable materials for future investigations, such as single-nucleotide polymorphism (SNP) and genome-wide association studies (GWAS).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14020300/s1.

Author Contributions: Conceptualization, A.T., K.H. and K.N.; methodology, F.D., A.T., K.H., S.D., B.E. and K.N.; software, A.T., F.D., K.N., H.B. and J.B.; validation, A.T., K.H., F.D., B.Y., K.N. and H.B.; formal analysis, A.T., K.H., F.D. and J.B.; investigation, F.D., A.T., K.H. and K.N.; resources, F.D., A.T., B.Y., B.E. and S.D.; data curation, A.T., K.H., F.D., K.N., H.B. and J.B.; writing—original draft preparation, F.D., A.T., K.H., B.E. and J.B.; writing—review and editing, A.T., K.H., F.D., B.Y., B.E., S.D., K.N., J.B. and H.B.; visualization, F.D., K.H., A.T. and J.B.; supervision, B.Y.; project administration, F.D., B.Y., K.H., A.T. and J.B.; funding acquisition, K.N. and H.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: All data supporting the conclusions of this article are included in this article.

Acknowledgments: This work is a molecular characterization part of Fatih Demirel's Ph.D. research dissertation and received financial assistance from the Scientific Research Projects Coordination Unit of Igdir University under project number "2017-FBE-D01".

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Shewry, P.R.; Hey, S.J. The contribution of wheat to human diet and health. *Food Energy Secur.* **2015**, *4*, 178–202. [CrossRef]
2. Shewry, P.R. Wheat. *J. Exp. Bot.* **2009**, *60*, 1537–1553. [CrossRef]
3. Adhikari, B.M.; Bajracharya, A.; Shrestha, A.K. Comparison of nutritional properties of Stinging nettle (*Urtica dioica*) flour with wheat and barley flours. *Food Sci. Nutr.* **2016**, *4*, 119–124. [CrossRef]
4. Sharma, S.; Schulthess, A.W.; Bassi, F.M.; Badaeva, E.D.; Neumann, K.; Graner, A.; Özkan, H.; Werner, P.; Knüpffer, H.; Kilian, B. Introducing beneficial alleles from plant genetic resources into the wheat germplasm. *Biology* **2021**, *10*, 982. [CrossRef]
5. Feldman, M.; Millet, E. The contribution of the discovery of wild emmer to an understanding of wheat evolution and domestication and to wheat improvement. *Isr. J. Plant Sci.* **2001**, *49*, 25–36. [CrossRef]
6. Özkan, H.; Brandolini, A.; Schäfer-Pregl, R.; Salamini, F. AFLP analysis of a collection of tetraploid wheats indicates the origin of emmer and hard wheat domestication in southeast Turkey. *Mol. Biol. Evol.* **2002**, *19*, 1797–1801. [CrossRef] [PubMed]
7. Luo, M.C.; Yang, Z.L.; You, F.; Kawahara, T.; Waines, J.; Dvorak, J. The structure of wild and domesticated emmer wheat populations, gene flow between them, and the site of emmer domestication. *Theor. Appl. Genet.* **2007**, *114*, 947–959. [CrossRef]
8. Ren, J.; Sun, D.; Chen, L.; You, F.M.; Wang, J.; Peng, Y.; Nevo, E.; Sun, D.; Luo, M.C.; Peng, J. Genetic diversity revealed by single nucleotide polymorphism markers in a worldwide germplasm collection of durum wheat. *Int. J. Mol. Sci.* **2013**, *14*, 7061–7088. [CrossRef] [PubMed]
9. Tadesse, W.; Amri, A.; Ogbonnaya, F.C.; Sanchez-Garcia, M.; Sohail, Q.; Baum, M. Wheat. In *Genetic and Genomic Resources for Grain Cereals Improvement*; Elsevier: Amsterdam, The Netherlands, 2016; pp. 81–124.
10. Türkoğlu, A.; Haliloğlu, K.; Mohammadi, S.A.; Öztürk, A.; Bolouri, P.; Özkan, G.; Bocianowski, J.; Pour-Aboughadareh, A.; Jamshidi, B. Genetic Diversity and Population Structure in Türkiye Bread Wheat Genotypes Revealed by Simple Sequence Repeats (SSR) Markers. *Genes* **2023**, *14*, 1182. [CrossRef] [PubMed]
11. Nadeem, M.A. Deciphering the genetic diversity and population structure of Turkish bread wheat germplasm using iPBS-retrotransposons markers. *Mol. Biol. Rep.* **2021**, *48*, 6739–6748. [CrossRef] [PubMed]
12. Baloch, F.S.; Alsaleh, A.; Shahid, M.Q.; Çiftçi, V.; Sáenz de Miera, E.L.; Aasim, M.; Nadeem, M.A.; Aktaş, H.; Özkan, H.; Hatipoğlu, R. A whole genome DArTseq and SNP analysis for genetic diversity assessment in durum wheat from central fertile crescent. *PLoS ONE* **2017**, *12*, e0167821. [CrossRef] [PubMed]
13. Benlioğlu, B.; Adak, M.S. Importance of crop wild relatives and landraces genetic resources in plant breeding programmes. *J. Exp. Agric. Int.* **2019**, *37*, 1–8. [CrossRef]
14. Ali, F.; Nadeem, M.A.; Habyarimana, E.; Yılmaz, A.; Nawaz, M.A.; Khalil, I.H.; Ercişli, S.; Chung, G.; Chaudhary, H.J.; Baloch, F.S. Molecular characterization of genetic diversity and similarity centers of safflower accessions with ISSR markers. *Braz. J. Bot.* **2020**, *43*, 109–121. [CrossRef]
15. Kocak, M.Z.; Kaysim, M.G.; Aydın, A.; Erdinc, C.; Kulak, M. Genetic diversity of flax genotypes (*Linum usitatissimum* L.) by using agro-morphological properties and molecular markers. *Genet. Res. Crop Evol.* **2023**, *70*, 2279–2306. [CrossRef]
16. Çayır, M.E.; Sevindik, E. Determining the genetic difference of some world cotton genotypes using iPbs (Inter-Primer Binding Sequences) retrotransposon markers. *J. Nat. Fibers* **2022**, *19*, 15213–15224. [CrossRef]
17. Demirel, S.; Demirel, F. Molecular identification and population structure of emmer and einkorn wheat lines with different ploidy levels using SSR markers. *Genet. Resour. Crop Evol.* **2023**, *71*, 363–372. [CrossRef]

18. Haliloğlu, K.; Türkoğlu, A.; Öztürk, A.; Niedbała, G.; Niazian, M.; Wojciechowski, T.; Piekutowska, M. Genetic diversity and population structure in bread wheat germplasm from Türkiye using iPBS-retrotransposons-based markers. *Agronomy* **2023**, *13*, 255. [CrossRef]
19. Karık, Ü.; Nadeem, M.A.; Habyarimana, E.; Ercişli, S.; Yildiz, M.; Yılmaz, A.; Yang, S.H.; Chung, G.; Baloch, F.S. Exploring the genetic diversity and population structure of Turkish laurel germplasm by the iPBS-retrotransposon marker system. *Agronomy* **2019**, *9*, 647. [CrossRef]
20. Song, H.; Dong, T.; Yan, X.; Wang, W.; Tian, Z.; Sun, A.; Dong, Y.; Zhu, H.; Hu, H. Genomic selection and its research progress in aquaculture breeding. *Rev. Aquac.* **2023**, *15*, 274–291. [CrossRef]
21. Vieira, E.A.; Carvalho, F.I.F.; Bertan, I.; Kopp, M.M.; Zimmer, P.D.; Benin, G.; Silva, J.A.G.; Hartwig, I.; Malone, G.; Oliveira, A.C. Association between genetic distances in wheat (*Triticum aestivum* L.) as estimated by AFLP and morphological markers. *Genet. Mol. Biol.* **2007**, *30*, 392–399. [CrossRef]
22. Nazarzadeh, Z.; Onsori, H.; Akrami, S. Genetic diversity of bread wheat (*Triticum aestivum* L.) genotypes using RAPD and ISSR molecular markers. *J. Genet. Res.* **2020**, *6*, 69–76.
23. Gurcan, K.; Demirel, F.; Tekin, M.; Demirel, S.; Akar, T. Molecular and agro-morphological characterization of ancient wheat landraces of turkey. *BMC Plant Biol.* **2017**, *17*, 171. [CrossRef]
24. Marić, S.; Bolarić, S.; Martinčić, J.; Pejić, I.; Kozumplik, V. Genetic diversity of hexaploid wheat cultivars estimated by RAPD markers, morphological traits and coefficients of parentage. *Plant Breed.* **2004**, *123*, 366–369. [CrossRef]
25. Heidari, P.; Etminan, A.; Azizinezhad, R.; Khosroshahli, M. Genomic variation studies in durum wheat (*Triticum turgidum* ssp. *durum*) using CBDP, SCoT and ISSR markers. *Indian J. Genet. Plant Breed.* **2017**, *77*, 379–386.
26. Elshafei, A.A.; Afiah, S.A.E.A.; Al-Doss, A.A.; Ibrahim, E.I. Morphological variability and genetic diversity of wheat genotypes grown on saline soil and identification of new promising molecular markers associated with salinity tolerance. *J. Plant Interact.* **2019**, *14*, 564–571. [CrossRef]
27. Wang, S.; Wong, D.; Forrest, K.; Allen, A.; Chao, S.; Huang, B.E.; Maccaferri, M.; Salvi, S.; Milner, S.G.; Cattivelli, L. Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. *Plant Biotechnol. J.* **2014**, *12*, 787–796. [CrossRef] [PubMed]
28. Tehseen, M.M.; Tonk, F.A.; Tosun, M.; Amri, A.; Sansaloni, C.P.; Kurtulus, E.; Yazbek, M.; AlSham'aa, K.; Ozseven, I.; Safdar, L.B. Genome-wide association study of resistance to PstS2 and Warrior races of *Puccinia striiformis* f. sp. *tritici* (stripe rust) in bread wheat landraces. *Plant Genome* **2021**, *14*, e20066. [CrossRef] [PubMed]
29. Nadeem, M.A.; Nawaz, M.A.; Shahid, M.Q.; Doğan, Y.; Comertpay, G.; Yıldız, M.; Hatipoğlu, R.; Ahmad, F.; Alsaleh, A.; Labhane, N. DNA molecular markers in plant breeding: Current status and recent advancements in genomic selection and genome editing. *Biotechnol. Biotechnolog. Equip.* **2018**, *32*, 261–285. [CrossRef]
30. Bennetzen, J.L. Transposable element contributions to plant gene and genome evolution. *Plant Mol. Biol.* **2000**, *42*, 251–269. [CrossRef] [PubMed]
31. Kalendar, R.; Antonius, K.; Smýkal, P.; Schulman, A.H. iPBS: A universal method for DNA fingerprinting and retrotransposon isolation. *Theo. Appl. Genet.* **2010**, *121*, 1419–1430. [CrossRef] [PubMed]
32. Zeinalzadehtabrizi, H.; Hosseinpour, A.; Aydin, M.; Haliloglu, K. A modified genomic DNA extraction method from leaves of sunflower for PCR based analyzes. *J. Biodivers. Environ. Sci.* **2015**, *7*, 222–225.
33. Yeh, F.C.; Yang, R.C.; Boyle, T. *POPGENE Software Package Version 1.31 for Population Genetic Analysis*; University of Alberta: Edmonton, AB, Canada, 1999.
34. Liu, K.; Muse, S.V. PowerMarker: An integrated analysis environment for genetic marker analysis. *Bioinformatics* **2005**, *21*, 2128–2129. [CrossRef]
35. Rohlf, F. NTSYS-pc: Numerical taxonomy (*Vitis vinifera* L.) varieties using morphological data and multivariate analysis system. Exeter Software and AFLP markers. *Elect. J. Biotechnol.* **2000**, *6*, 37–45.
36. Mantel, N. The detection of disease clustering and a generalized regression approach. *Cancer Res.* **1967**, *27*, 209–220.
37. Eren, B.; Keskin, B.; Demirel, F.; Demirel, S.; Türkoğlu, A.; Yilmaz, A.; Haliloğlu, K. Assessment of genetic diversity and population structure in local alfalfa genotypes using iPBS molecular markers. *Genet. Resour. Crop Evol.* **2023**, *70*, 617–628. [CrossRef]
38. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of population structure using multilocus genotype data. *Genetics* **2000**, *155*, 945–959. [CrossRef]
39. Evanno, G.; Regnaut, S.; Goudet, J. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Mol. Ecol.* **2005**, *14*, 2611–2620. [CrossRef]
40. Earl, D.A.; VonHoldt, B.M. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conser. Genet. Res.* **2012**, *4*, 359–361. [CrossRef]
41. Abbasi Holasou, H.; Rahmati, F.; Rahmani, F.; Imani, M.; Talebzadeh, Z. Elucidate genetic diversity and population structure of bread wheat (*Triticum aestivum* L.) cultivars using IRAP and REMAP markers. *J. Crop Sci. Biotechnol.* **2019**, *22*, 139–151. [CrossRef]
42. Amom, T.; Nongdam, P. The use of molecular marker methods in plants: A review. *Int. J. Curr. Res. Rev.* **2017**, *9*, 1–7.
43. Kumar, P.; Sharma, V.; Sanger, R.; Kumar, P.; Yadav, M.K. Analysis of molecular variation among diverse background wheat (*Triticum aestivum* L.) genotypes with the help of ISSR markers. *Int. J. Chem. Stud.* **2020**, *8*, 271–276. [CrossRef]

44. Alshehri, M.; Alzahrani, O.; Aziza, A.; Alasmari, A.; Ibrahim, S.; Bahattab, O.; Osman, G.; Alshamari, A.; Alduaydi, S. Correlation and genetic analyses of different characteristics in Saudi Arabian wheat reveal correlation networks and several trait-associated markers. *J. Anim. Plant Sci.* **2020**, *30*, 1486–1497.
45. Çifçi, E.A.; Yağdi, K. Study of genetic diversity in wheat (*Triticum aestivum*) varities using random amplified polymorphic DNA (RAPD) analysis. *Turk. J. Field Crop.* **2012**, *17*, 91–95.
46. Yirgu, M.; Kebede, M.; Feyissa, T.; Lakew, B.; Woldeyohannes, A.B.; Fikere, M. Single nucleotide polymorphism (SNP) markers for genetic diversity and population structure study in Ethiopian barley (*Hordeum vulgare* L.) germplasm. *BMC Genom. Data* **2023**, *24*, 7. [CrossRef]
47. Najaphy, A.; Parchin, R.A.; Farshadfar, E. Evaluation of genetic diversity in wheat cultivars and breeding lines using inter simple sequence repeat markers. *Biotechnol. Biotechnol. Equip.* **2011**, *25*, 2634–2638. [CrossRef]
48. Khalid, S.; Shahid, M.; Natasha Shah, A.H.; Saeed, F.; Ali, M.; Qaisrani, S.A.; Dumat, C. Heavy metal contamination and exposure risk assessment via drinking groundwater in Vehari, Pakistan. *Environ. Sci. Pollut. Res.* **2020**, *27*, 39852–39864. [CrossRef]
49. Abo-Youssef, M.I.; Bahgt, M.M. Assessment of genetic diversity of diverse rice genotypes using Agro-Physiological and molecular characterization under water deficit conditions. *EJSS* **2023**, *63*, 503–524.
50. Palaz, E.B.; Demirel, F.; Adali, S.; Demirel, S.; Yilmaz, A. Genetic relationships of salep *Orchid* species and gene flow among *Serapias vomeracea* × *Anacamptis morio* hybrids. *Plant Biotechnol. Rep.* **2023**, *17*, 315–327. [CrossRef]
51. Mohammadi, S.A.; Prasanna, B. Analysis of genetic diversity in crop plants—Salient statistical tools and considerations. *Crop Sci.* **2003**, *43*, 1235–1248. [CrossRef]
52. Nasri, S.; Abdollahi Mandoulakani, B.; Darvishzadeh, R.; Bernousi, I. Retrotransposon insertional polymorphism in Iranian bread wheat cultivars and breeding lines revealed by IRAP and REMAP markers. *Biochem. Genet.* **2013**, *51*, 927–943. [CrossRef]
53. Saeidi, H.; Rahiminejad, M.R.; Heslop Harrison, J. Retroelement insertional polymorphisms, diversity and phylogeography within diploid, D-genome *Aegilops tauschii* (Triticeae, Poaceae) sub-taxa in Iran. *Ann. Bot.* **2008**, *101*, 855–861. [CrossRef] [PubMed]
54. Beharav, A.; Maras, M.; Kitner, M.; Šuštar Vozlič, J.; Sun, G.; Doležalová, I.; Lebeda, A.; Meglič, V. Comparison of three genetic similarity coefficients based on dominant markers from predominantly self-pollinating species. *Biol. Plant.* **2010**, *54*, 54–60. [CrossRef]
55. Shi, J.; Lai, J. Patterns of genomic changes with crop domestication and breeding. *Curr. Opin. Plant Biol.* **2015**, *24*, 47–53. [CrossRef] [PubMed]
56. Scheben, A.; Edwards, D. Towards a more predictable plant breeding pipeline with CRISPR/Cas-induced allelic series to optimize quantitative and qualitative traits. *Curr. Opin. Plant Biol.* **2018**, *45*, 218–225. [CrossRef]
57. Borrelli, V.M.; Brambilla, V.; Rogowsky, P.; Marocco, A.; Lanubile, A. The enhancement of plant disease resistance using CRISPR/Cas9 technology. *Front. Plant Sci.* **2018**, *9*, 1245. [CrossRef]