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ORIGINAL ARTICLE

Deciphering the genetic diversity and population structure of Turkish bread wheat germplasm using iPBS-retrotransposons markers

Muhammad Azhar Nadeem[1]

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Abstract

Background Research activities aiming to investigate the genetic diversity are very crucial because they provide information for the breeding and germplasm conservation activities. Wheat is one of the most important cereal crops globally by feeding more than a third of the human population around the world.

Methods and results During present investigation, a total of 74 Turkish bread wheat accessions (54 landraces and 20 cultivars) were used as plant material and iPBS-retrotransposons marker system was used for the molecular characterization. 13 polymorphic primers used for molecular characterization resulted a total of 152 bands. Range of calculated diversity indices like polymorphism information content (0.11–0.702), effective numbers of alleles (1.026–1.526), Shannon's information index (0.101–0.247) and gene diversity (0.098–0.443) confirmed higher genetic variations in studied germplasm. Bread wheat landraces reflected higher genetic variations compared to commercial cultivars. Analysis of molecular variance resulted that higher (98%) genetic variations are present within populations. The model-based structure algorithm separated 74 bread wheat accessions in to two populations. Diversity indices based on structure evaluated population's revealed population B as a more diverse population. The principal coordinate analysis and neighbor-joining analysis separated 74 bread wheat accessions according to their collection points. Genetic distance for 74 Turkish bread wheat accessions explored Bingol and Asure accessions as genetically diverse that can be used as parents for breeding activities.

Conclusions The extensive diversity of bread wheat in Turkish germplasm might be used as genetic resource for the exhaustive wheat breeding program. For instance, accessions Bingol and Asure were found genetically diverse and can be used as parents for future breeding activities.

Keywords *Triticum aestivum* L. · Jumping elements · Molecular characterization · Turkey

Introduction

The world is facing a big problem of food scarcity due to climate change and rapidly increasing world population. Khush et al. [1] stated that nearly 800 million people from the developing countries go to bed hungry. World population is increasing with rapid pace and estimated to reach 10 billion by 2050. Due to rapid increase in the world population and continuous changes in climatic conditions, there is a need to boost up world food production to meet food demands in 2050 [2, 3].

Wheat (*Triticum aestivum* L.) is hexaploid ($2n = 6x = 42$) is an important cereal crop belonging to *Poaceae* family and serving a source a food for millions of people [4, 5]. It is believed that bread wheat was originated through two polyploidization events between *Triticum urartu* (AA genome) and an *Aegilops speltoides* related species (BB genome) nearly 0.5 million years ago (hereafter Ma), resulting in the formation of *Triticum turgidum* ssp. *diccocoides* [6, 7]. Finally, hybridization between *Triticum turgidum* ssp. Durum (AABB genome) and *Aegilops tauschii* (DD genome) nearly 10,000 years ago in Fertile Crescent, in a region that nowadays comprises Northern Iran resulted in the formation of modern day hexaploid bread wheat (AABBDD) genome [6–8]. During 2019, wheat was cultivated globally on an area of 215,901,958 ha with a production of 765,769,635 tones [9]. Fertile Crescent which includes part of present-day Turkey is considered the origin and domestication center

✉ Muhammad Azhar Nadeem
azharjoiya22@gmail.com

[1] Faculty of Agricultural Sciences and Technologies, Sivas University of Science and Technology, Sivas, Turkey

of wheat and its progenitors [10]. During 2019, wheat was cultivated globally on an area of 6,831,854 ha with a production of 19,000,000 tones [9]. As Turkey is a part of Fertile Crescent, therefore it is very important to explore and understand the genetic diversity in Turkish wheat and its wild relatives genetic resources for wheat improvement program [11]. Germplasm characterization is considered prerequisite for breeding activities as it facilitate novel genetic variations to the breeders that can be used for marker-assisted breeding [12]. Previous report confirmed that domestication, human selection, and breeding activities for improved traits resulted in genetic erosin and lowered the diversity wheat gene pool [13]. Therefore, studies regarding the assessment of genetic variations in wheat are very important for future breeding activities. Morphological and molecular markers are two widely used approaches for the characterization of germplasm. However, DNA based markers are more trustable and reproducible and not influenced by environmental factors compared to morphological markers [14].

Advancement in molecular markers techniques revolutionized the breeding activities [14]. Among these, retrotransposons are genetic elements having ability to copy their numbers, change their location and constitute major components of most eukaryotic genomes [15]. Long terminal repeat (LTR) and non-LTR retrotransposons are two major groups of retrotransposons. LTR- retrotransposons are in prevalence and more active in plants compared to non-LTR retrotransposons [16, 17]. However, limitations in both LTR and non-LTR retrotransposons leads the scientific community to develop inter primer binding site (iPBS) marker system [17]. Kalendar et al. [17] proposed iPBS-retrotransposons as a universal marker that can be used for the characterization of both animal and plant species. iPBS-retrotransposons markers has been used for the molecular characterization, phylogenetic and evolutionary study in various crop plants [18–20]. Previously, different molecular markers has been used for the molecular characterization of wheat germplasm [21–23]. However, there is scarcity of information about the characterization of bread wheat germplasm using iPBS-retrotransposons. Therefore, current study aimed to characterize Turkish bread wheat germplasm for the assessment of genetic diversity and to explore its population structure.

Materials and methods

Plant material and DNA isolation

74 bread wheat accessions including a total of 54 landraces and 20 commercial cultivars were used for the molecular characterization (Table 1). These landraces were collected from 14 provinces of Turkey (Fig. 1). To isolate the genomic DNA, all bread wheat accessions were sown in the greenhouse and their fresh and young leaves were harvested. The DNA extraction was achieved using CTAB protocol [24] and a specific protocol recommended by Diversity Arrays Technology (available at https://www.diversityarrays.com/orderinstructions/plant-dnaextraction-protocol-for-dart/). Quantification of isolated DNA samples was performed using 0.8% agarose gel and NanoDrop (DS11 FX, DeNovix Inc., Wilmington, DE, USA). 5 ng/µL was prepared as a final concentration for further polymerase chain reaction (PCR) analysis.

iPBS-retrotransposons PCR amplifications

Initially, 75 iPBS primers were screened on randomly selected 10 bread wheat accessions. Among these 75 screened primers, 13 most polymorphic primers were evaluated for final PCR amplification of all 74 bread wheat accessions (Table 2). PCR amplification was executed according to the methodology of Kalendar et al. [17]. After PCR amplification, PCR product was run on 2% (w/v) agarose gel having TBE buffer (0.5×) at a stable voltage of 120 V for 220 min. Staining of gel was performed using ethidium bromide and graphics were taken through a UV Imager Gel Doc XR+system (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Statistical analysis

As a dominant marker system, scoring was performed in binary fashion; 1 or 0 representing the presence and absence of a band respectively. Various diversity parameters like gene diversity (He) Shannon's information index (I) and effective alleles number (Ne) were investigated through Popgene ver. 1.32 [25]. The Nei's genetic distance among 74 Turkish bread wheat accessions was calculated using Popgene ver. 1.32 [25]. To explore genetic variations between landraces and cultivars, various diversity indices were also calculated using GenAlExV6.5 [26] software. The polymorphism information contents (PIC) was found using a formula i.e. $PIC = 2fi\ (1 - fi)$, given by Roldán-Ruiz et al. [27]. Here fi represents the frequency of present loci of a molecular marker while $(1 - fi)$ represents the frequency of absent loci. Principal coordinate analysis (PCoA) and analysis of molecular variance (AMOVA) were calculated through GenAlExV6.5 [26] software. To explore the genetic relationship among 74 Turkish bread wheat accessions, neighbor joining analysis was performed using R statistical software. Structure software was used to explore the population structure of Turkish bread wheat germplasm (Pritchard et al. [28]). The favorable numbers of clusters (K subpopulations) were estimated (1–10) by repeating analysis three time according to the

Table 1 Passport data of Turkish bread wheat accessions used in this study

Sr. No.	Accession Name	Accession Sr. No.	Sr. No.	Accession Name	Accession Sr. No.
1	Agri1	TR32743	41	Nigde3	TR81545
2	Kars1	TR32862	42	Bitlis5	TR46772
3	Kayseri	TR32034	43	Bitlis6	TR46774
4	Yozgat	TR35147	44	Van5	TR46774
5	Erzurum1	TR45332	45	Erzurum6	TR45359
6	Erzurum2	TR45339	46	Agri3	TR45380
7	Erzurum3	TR45334	47	Agri4	TR45382
8	Agri2	TR45376	48	Van5	TR45399
9	Van1	TR45359	49	Van6	TR45403
10	Van2	TR45409	50	Elazagi4	TR46845
11	Bitlis1	TR46751	51	Kars	TR46850
12	Bitlis2	TR46752	52	Van7	TR45405
13	Van3	TR46755	53	Van8	TR47997
14	Elazagi1	TR46839	54	Kars2	TR48224
15	Elazagi2	TR46841	55	Kirmizibugday*	
16	Elazagi3	TR46844	56	Ceyhan99*	
17	Yozgat1	TR45308	57	Bezostaya*	
18	Erzurum4	TR45331	58	Gerek79*	
19	Bitlis3	TR47952	59	Nenehatun*	
20	Bitlis4	TR47957	60	Cumhuriyet75*	TR40990
21	Erzincan1	TR48045	61	Kasifbey95*	TR62161
22	Erzincan2	TR48050	62	Alibey*	TR80704
23	Van4	TR46769	63	Ege88*	TR57777
24	Erzurum5	TR49109	64	Solen2002*	TR80706
25	Sivas1	TR53299	65	Korosu90*	
26	Sivas2	TR53313	66	Kirik*	
27	Sivas3	TR53318	67	Alparslan*	
28	Sivas4	TR53327	68	Jan3*	
29	Erzincan3	TR53329	69	Asure*	
30	Yozgat2	TR53862	70	Ayyildiz*	
31	Yozgat3	TR53863	71	Topbas*	
32	Eskisehir1	TR55181	72	Lancer*	
33	Eskisehir2	TR55182	73	Silverstar*	
34	Ankara1	TR80983	74	Seri82*	
35	Ankara2	TR81100			
36	Ankara3	TR81197			
37	Ankara4	TR81279			
38	Nigde1	TR81375			
39	Nigde2	TR81549			
40	Bingol1	TR81160			

*Commercial cultivars

report of Evanno et al. [29]. During each run, the burn-in and Markov Chain Monte Carlo (MCMC) were set to 50,000 each, and iterations were set to 10. Later, structure evaluated results were processed with STRUCTURE HARVESTER v.0.9.94 [30] to investigate most favorable K value. The pophelper an R package was used to visualize the most favorable ΔK [31].

Results

During this study, 13 iPBS-retrotransposons primers were used for the molecular characterization of Turkish bread wheat germplasm. These 13 primers resulted 152 bands and 11.69 bands were average bands/primer (Table 3).

Fig. 1 Collection provinces of Turkish bread wheat germplasm

Table 2 Characteristics of iPBS-retrotransposons primers used for the molecular characterization of bread wheat germplasm

Primer	Sequence (5′-3′)	Annealing temperature (°C)
2074	GCTCTGATACCA	49.6
2077	CTCACGATGCCA	55
2095	GCTCGGATACCA	53.7
2230	TCTAGGCGTCTGATACCA	52.9
2237	CCCCTACCTGGCGTGCCA	55
2246	ACTAGGCTCTGTATACCA	49
2252	TCATGGCTCATGATACCA	52
2255	GCGTGTGCTCTCATACCA	50
2257	CTCTCAATGAAAGCACCA	50
2374	CCCAGCAAACCA	53
2376	TAGATGGCACCA	52
2381	GTCCATCTTCCA	50
2390	GCAACAACCCCA	56

iPBS-2257 and iPBS-2257 produced maximum (16) bands, while minimum (4) bands were yielded with iPBS-2246. Among 152, 111 (73.2%) bands were found polymorphic, while 8.54 were average bands/primer. iPBS-2095 was found most polymorphic primer as it produced maximum numbers of polymorphic bands. The iPBS-2095 and iPBS-2381 primers showed maximum (100%) polymorphism. PIC value ranged 0.702 to 0.11 for iPBS-2074 and iPBS-2376 respectively, and mean PIC value was 0.42. The iPBS-2374 and iPBS-2376 produced maximum (1.526) and minimum (1.026) effective number of alleles respectively, while 1.312 was mean effective number of alleles during this study. Shannon's information index ranged 0.101 for iPBS-2376 to 0.247 for iPBS-2374 and mean Shannon's information index during this study was 0.165. Mean gene diversity was 0.256, while iPBS-2376 and iPBS-2374 resulted minimum (0.098) and maximum (0.443) gene diversity. Mean Nei's genetic distance was 0.190, while maximum and minimum genetic distance was 0.427 (Bingol and Asure) and 0.04 (Van5 and Agri4). Various diversity indices were also calculated among bread wheat landraces and cultivars to explore the level of genetic variations (Table 4). Turkish bread wheat landraces reflected higher polymorphism (66.45%) and other calculated diversity indices compared to cultivars. Results of AMOVA reported the existence of higher (98%) genetic variation within population compared to among the populations (2%) (Table 5).

The genetic structure of Turkish bread wheat germplasm was separated into two groups as proposed by ΔK peak at K = 2 constructed in the structure harvester analysis (Fig. 2). The model-based structure algorithm separated 74 bread wheat accessions in to two populations on the basis of their collection points (Fig. 3). Population A clustered a total of 58 bread wheat accessions, while 16 accessions were present in population B. Various diversity indices and AMOVA was also calculated among structure evaluated populations (Table 6). Results showed that population B has more genetic variations compared to population A. The AMOVA also revealed the existence of higher genetic variations within population (92%) compared to among the populations (8%). The neighbor-joining analysis separated 74 bread wheat accessions into three population on the basis of their collection points (Fig. 4). The PCoA analysis strengthen the clustering of model-based structure algorithm by separating the 74 bread wheat accessions into two populations (Fig. 5).

Table 3 Diversity parameters in Turkish bread wheat germplasm using iPBS-retrotransposons marker system

Primer	Total bands	PB	P%	PIC	Ne	I	He
2074	9	4	44.4	0.702	1.164	0.117	0.182
2077	10	6	60.0	0.680	1.197	0.125	0.192
2095	13	13	100.0	0.442	1.289	0.196	0.326
2230	7	5	71.4	0.511	1.422	0.247	0.370
2237	15	5	33.3	0.138	1.104	0.109	0.114
2246	4	4	100	0.395	1.144	0.124	0.242
2252	16	14	87.5	0.405	1.269	0.179	0.292
2255	11	9	81.8	0.390	1.185	0.124	0.216
2257	16	8	50.0	0.260	1.209	0.119	0.179
2374	15	13	86.7	0.669	1.526	0.298	0.443
2376	10	5	50.0	0.11	1.026	0.101	0.098
2381	10	10	100.0	0.475	1.312	0.215	0.359
2390	16	15	93.8	0.380	1.325	0.196	0.311
Total	152	111					
Mean	11.69	8.54	73.76	0.42	1.244	0.165	0.256

PB Polymorphic bands, *P%* Polymorphism percentage, *PIC* polymorphism information content, *Ne* effective numbers of alleles, *I* Shannon's information index, *He* gene diversity

Table 4 Diversity parameters in Turkish bread wheat landraces and cultivars using iPBS-retrotransposons marker system

	Na	Ne	I	He	uHe	%P
Landraces	1.658	1.368	0.330	0.219	0.221	66.45%
Cultivars	1.533	1.356	0.298	0.203	0.209	54.61%

Na No. of different alleles, *Ne* effective number of alleles, *I* Shannon's information index, *He* expected heterozygosity, *uHe* unbiased expected heterozygosity, *%P* polymorphism percentage

Table 5 The AMOVA results exploring genetic variations in Turkish bread wheat landraces and cultivars using iPBS-retrotransposons marker system

	df	SS	MS	Est. Var	%variations
Among populations	1	4.894	4.894	0.058	2
Within populations	72	230.375	3.200	3.200	98
Total	73	235.269		3.258	100

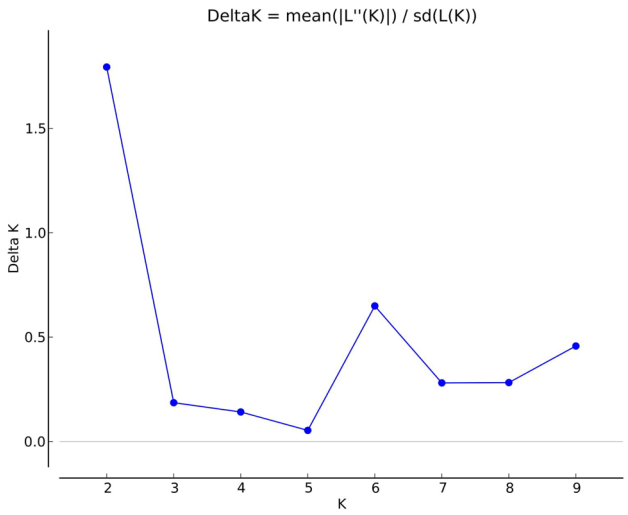

Fig. 2 Delta K value proposing the presence of two populations of Turkish bread wheat germplasm using iPBS-retrotransposons marker system

Discussion

A reasonable studies have been documented for the characterization of bread wheat germplasm and its wild relatives using various types of molecular markers [21–23]. Regarding to retrotransposons based markers Demirel [32], used iPBS-retrotransposons marker for the molecular characterization of emmer and durum wheat. Queen et al. [33] used SSAP marker for linkage and genetic diversity analysis in bread wheat and its wild relatives. Similarly, Holasou et al. [21] used IRAP and REMAP markers for the molecular characterization of 49 Iranian bread wheat cultivars. However to best of knowledge, iPBS-retrotransposons

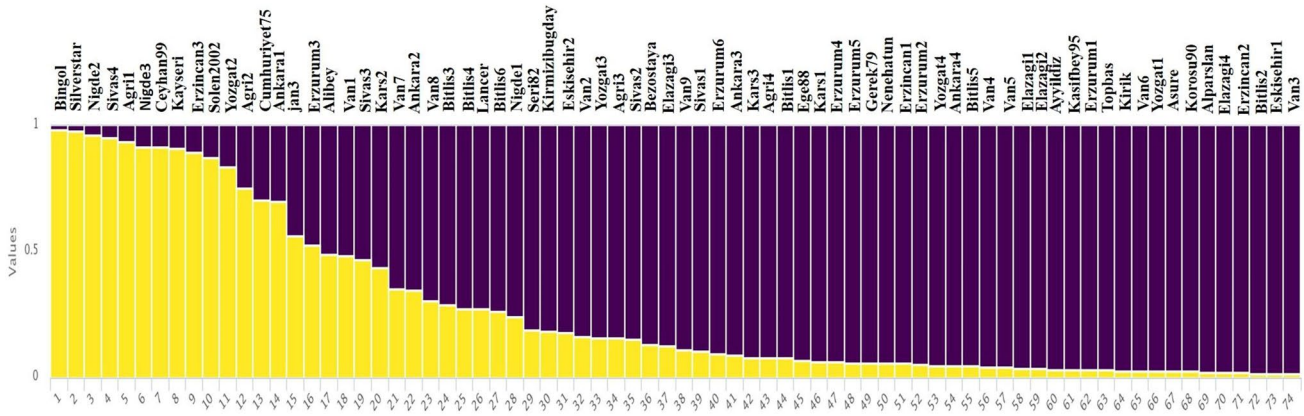

Fig. 3 Population structure of Turkish bread wheat germplasm using iPBS-retrotransposons marker system

Table 6 Diversity indices and AMOVA among structure based populations using iPBS-retrotransposons marker system

	Na	Ne	I	He	uHe	Polymorphism (%)
Population A	1.513	1.300	0.261	0.174	0.176	51.97
Population B	1.632	1.437	0.366	0.248	0.257	66.45
Source	df	SS	MS		Est. Var	%
Among Pops	1	69.771	69.771		1.789	8
Within Pops	72	1513.554	21.022		21.022	92
Total	73	1583.324			22.811	100

Na No. of different alleles, *Ne* effective number of alleles, *I* Shannon's information index, *He* expected heterozygosity, *uHe* unbiased expected heterozygosity, *%P* polymorphism percentage

markers are not used for the characterization of bread wheat germplasm.

During this study, 13 iPBS-retrotransposons primers yielded a total of 152 bands, among which 111 were found polymorphic (Table 3). Total and polymorphic bands reported in this study were higher than Nazarzadeh et al. [34] using RAPD and ISSR markers Kumar et al. [35], using ISSR marker Alshehri et al. [36], using SCoT and ISSR primers and Çifçi and Yağdi [37] using RAPD markers. The range and mean PIC value reported herein was found higher than earlier studies of Kumar et al. [35] using ISSR markers El-Sherbeny et al. [38], using ISSR markers and AL-Tamimi and AL-Janabi [39] using RAPD and ISSR markers. The resulted mean and range of effective number of alleles was higher than the Kumar et al. [35]. It was observed that iPBS-retrotransposons primers resulting the less number of alleles also resulted in low gene diversity. Similarly, higher gene diversity was observed for the primers producing higher alleles. This pattern was found similar with the Kumar et al. [35]. Mean gene diversity and Shannon's information index observed in present report was higher than the Carvalho et al. [40]. Presence of higher values for various diversity indices in this study might be due to differences in germplasm and the nature of molecular marker. iPBS-retrotransposons marker system has been found highly reproducible and its universal nature has been already proven in various studies [18, 41]. Therefore, this marker system should be preferred for the molecular characterization of bread wheat germplasm compared to other dominant marker systems.

During this study, bread wheat landraces and cultivars were used as a plant material. Therefore, calculated diversity indices among landraces and cultivars showed the presence of higher genetic variations in landraces compared to cultivars. These results were in line with previous studies as they also reported the existence of higher genetic variations in wheat landraces compared to their cultivars [42, 43]. The AMOVA results revealed that maximum genetic variations in Turkish bread wheat germplasm are present within population. Results of AMOVA were also supported by previous studies as they also revealed higher genetic variations within populations [44, 45]. The Nei's genetic distance revealed Bingol and Asure as genetically distinct accessions. Arystanbekkyzy et al. [41] stated that genetically distinct accessions are can be helpful to start breeding activities for favorable

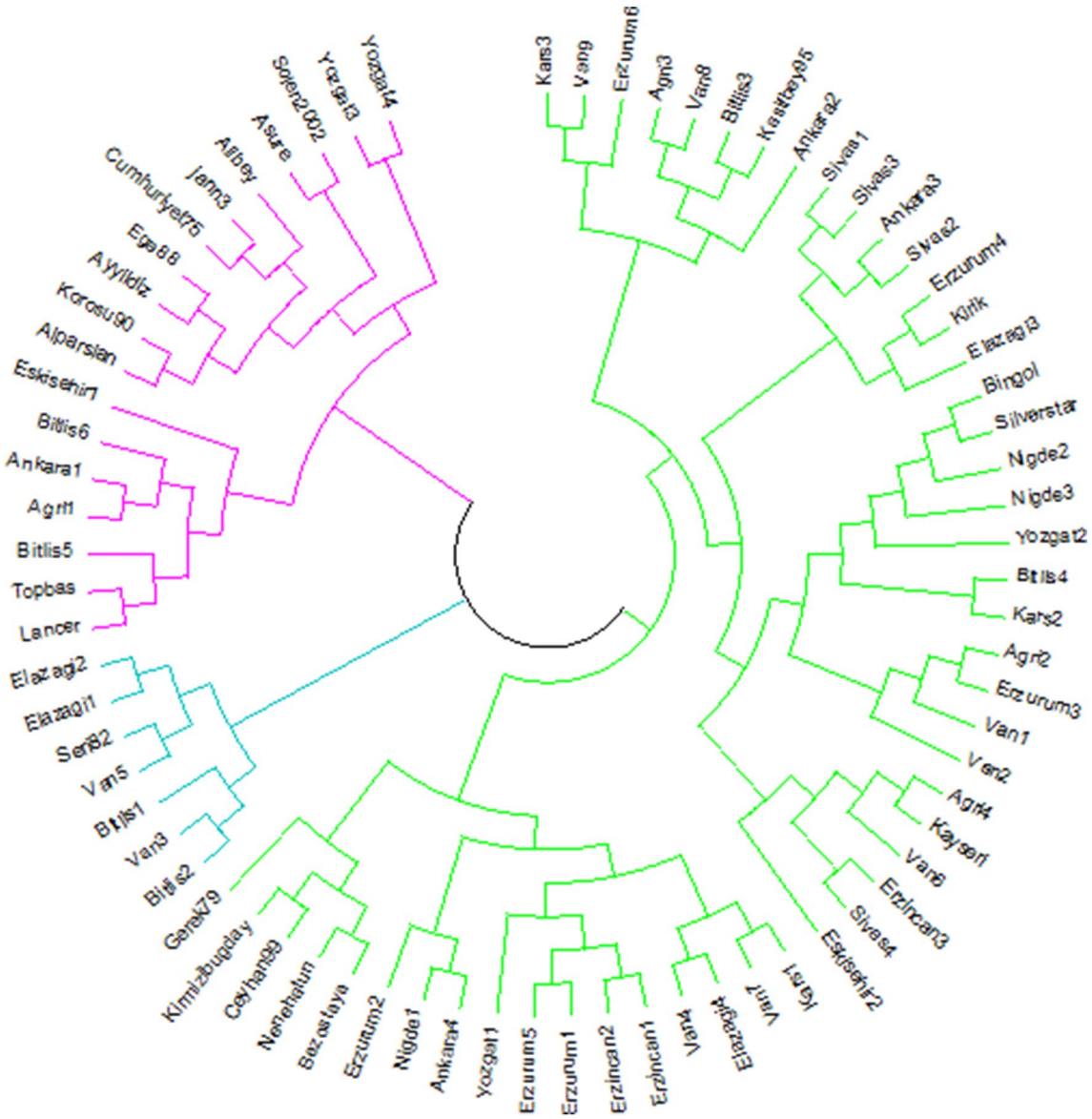

Fig. 4 The neighbor-joining analysis based clustering of Turkish bread wheat germplasm using iPBS-retrotransposons marker system

traits. Therefore, Bingol and Asure accessions can be used for future bread wheat breeding.

The model-based structure algorithm grouped 74 bread wheat accessions into two populations on the basis of their collection points (Fig. 3). Population A was found larger than population B by accounting 78.37% (58 accessions) accessions. Population B accounted a total of 16 accessions and 6 of these were commercial cultivars. The remaining 10 commercial cultivars were present in population A. It can be seen in structure results that accessions belonging to same province or their neighbor province were present showed similarity with each other. For example, accessions from Erzurum showed genetic similarity with Kars and similar was the case with the accessions from Bitlis and Van. It was also observed that accessions from east and north east provinces of Turkey were present in population B by making it a diverse population and showing their genetic similarity with each other's. The neighbor-joining analysis grouped studied germplasm into three populations mainly on the basis of their collection points (Fig. 4). Population C was found larger than rest of the populations and clustered a total of 49 accessions. A total of 11 cultivars were grouped in population A, while population B and C accounted a total of 1 and 8 cultivars respectively. The neighbor-joining analysis showed admixture of accessions because accessions from various provinces were grouped under the same sub-groups. Therefore, preference was given to structure clustering because structure algorithm has been proven more trustable

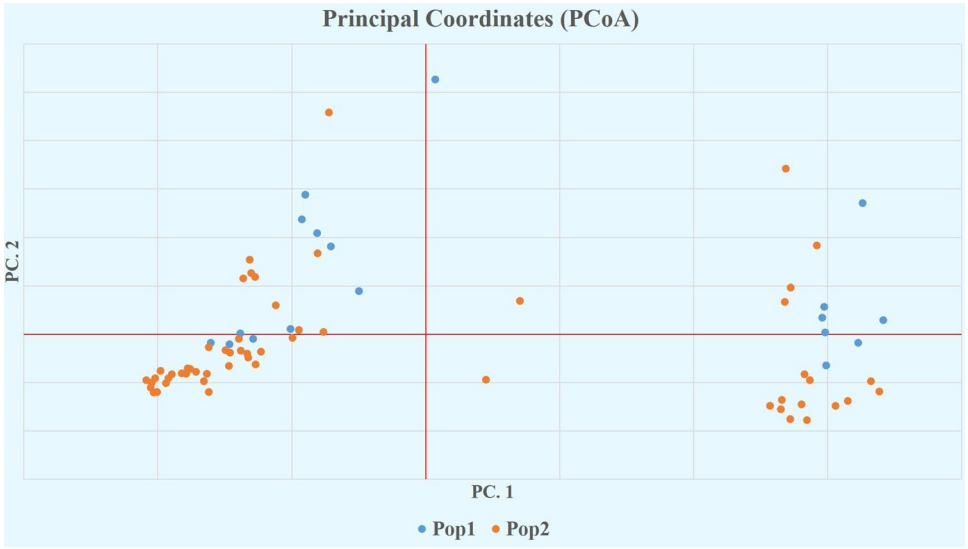

Fig. 5 Principal coordinate analysis (PCoA) of Turkish bread wheat germplasm using iPBS-retrotransposons marker system

and much informative compared to other clustering algorithms [46, 47]. Different diversity parameters were also calculated for structure evaluated populations that revealed the existence of higher genetic variations in population B. The AMOVA analysis for structure evaluated population also confirmed the presence of higher genetic variations within populations compared to among the populations. Thus, it is stated that Turkish bread wheat germplasm has great level of genetic variations within the population that can be helpful for the breeding of this crop in future. The PCoA analysis supported the clustering of model-based structure algorithm and separated the Turkish bread wheat germplasm into two populations (Fig. 5).

Conclusion

This study provided a deep insight about genetic variations in Turkish bread wheat germplasm using iPBS-retrotransposons marker system. The Bingol and Asure were found genetically most diverse accessions and should be used for future breeding activities. Results of AMOVA explored higher genetic variations within populations compared to among the populations. Population A from structure clustering was found more diverse and accessions belonging to this population should be considered for future wheat breeding. The model-based structure algorithm and PCoA separated the studied germplasm into population mainly on the basis of their collection points. Present study also confirmed the applicability and universal nature of iPBS-retrotransposons markers that can be used for the investigation of genetic diversity of any crop.

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Author contributions MAN conceptualized and established the methodology for this study, also performed molecular characterization of bread wheat germplasm, statistical analysis, and wrote the manuscript.

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Data availability All data needed to conduct this study is provided within the manuscript.

Declarations

Conflict of interest The author confirm that this article content has no conflict of interest.

Consent to publish Author read the manuscript and showed his willingness to publish this study.

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