

Genetic Diversity of Bread Wheat Genotypes Grown in Different Landscapes Using SSR Marker Analysis

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Abstract. Azerbaijan, a primary gene center, contains a rich diversity of wheat at different altitudes. Studying this diversity is crucial for improving adaptability and resilience to climate change in high altitude regions. A total of 158 bread wheat accessions were analyzed using SSR markers, following CTAB and PCR-based DNA extraction, fragment analysis on an ABI 3130xl Genetic Analyzer and subsequent statistical evaluation. Thirty-five alleles were identified, ranging from 4 to 16 per primer, with an average value of 8.8. The expected heterozygosity (He) ranged from 0.453 to 0.858, with *barc212* showing the highest value, while the polymorphism information content (PIC) ranged from 0.392 to 0.846. *Var. ferrugineum* showed the highest genetic variability, while *var. albidum* showed the least. The accessions were grouped into three main clusters in the dendrogram. Three accessions from Hajikend (collected from 1258 m) formed distinct clusters with genetic distances ranging from 0.33 to 0.5. Similarly, accessions from Kankendi (700-800 m) showed a genetic distance of 0.67 between clusters. Overall, SSR marker analysis of local bread wheat revealed significant polymorphism, with accessions from high altitude contributing to the diversity. These results provide valuable information for selecting different parental combinations in breeding programs and hybridization efforts, enhancing crop sustainability and food security.

Keywords: bread wheat, botanical variety, SSR, biodiversity, polymorphism, cluster.

1 Introduction

Azerbaijan is recognized as one of the world's primary gene centers, distinguished by its rich diversity of cereal plants and their wild ancestors, which thrive across various altitudes and landscapes [1, 2]. The preservation and study of this unique biodiversity are essential for the development of high-quality crop varieties that can withstand abiotic and biotic stress factors, which are increasingly relevant in the face of climate change and environmental degradation [3-6].

For over a century, wheat has been the central focus of agricultural and biological research, with significant attention given to its evolution, genetics, biodiversity, and

ecology [7-9]. Among the various wheat species, bread wheat (*Triticum aestivum* L.) is particularly noteworthy for its high nutritional value and widespread cultivation.

As climate warming progresses, it is facilitating the cultivation of wheat on previously unused land at higher latitudes [10]. Concurrently, rising temperatures are extending the growing season in mountainous regions, prompting farmers to shift wheat cultivation to higher elevations, a trend observed across various crops [11]. Wheat thrives in diverse environments, ranging from sea level to elevations exceeding 3000 meters above sea level (masl), and has even been documented at altitudes of 4570 masl in Tibet. Furthermore, it has been revealed that Tibetan wheat accessions possess haplotypes adapted to high-altitude conditions, allowing them to respond effectively to challenging environmental pressures [12].

However, wheat yields vary among countries at higher latitudes [13] due to the uneven rate of climate warming, alongside differences in precipitation and soil characteristics. Additionally, cultivation altitude affects not only yield but also protein content and volatile compound profiles, with elevation driving variety-specific adaptations in volatile synthesis [14]. Therefore, exploring greater genetic diversity in wheat is essential to enhance adaptability and resilience in the face of changing environmental conditions at high altitudes, ultimately supporting sustainable agricultural practices in these challenging environments.

Unlike tetraploid wheats, bread wheat possesses three genomes (AABBDD) and is a staple in diets worldwide [15-18]. Despite the complexities posed by the hexaploid genome of *T. aestivum*, successful sequencing efforts have revealed its remarkable size, with approximately 70% comprised of repetitive DNA sequences. The complete genome and coding DNA of the bread wheat variety "Chinese Spring" (CS42) were first sequenced in 2012 by Brenchley et al. using a Roche 454 Next Generation Sequencer [19]. Analysis of this genomic data has identified a total of 124201 genes within the bread wheat genome. Research indicates that the A and B genomes originated from a common ancestral source approximately 7 million years ago, with the D genome integrating into these genomes 1-2 million years later [20, 21]. Additionally, investigations into the wheat transcriptome have uncovered 46487 genes associated with endosperm development [22]. It was determined that high-altitude environments can induce significant genomic modifications in wheat [12].

The discovery of numerous markers related to various morphological traits through genomic sequencing has advanced breeding programs designed to improve the resilience and productivity of many crops [23, 24], including wheat. The initial SSR map of bread wheat, comprising 279 loci, was established in 1998 [25]. A subsequent map developed six years later expanded to include 1235 SSR loci, covering 2569 cM of the genome [26]. Numerous studies have demonstrated that hexaploid wheat exhibits lower genetic variation compared to diploid species [27]. It is posited that during the evolution of bread wheat, certain SSR loci from diploid genomes may have been lost, or the primer binding sites may have mutated, diminishing the transferability of SSR markers from related species. Thus, the investigation of microsatellite regions in bread wheat, the identification of polymorphic loci, and their application in future breeding and genetic research are of paramount importance.

The main objective of this study is to evaluate the genetic diversity of 158 bread wheat accessions using SSR markers. Most of these accessions were collected from various elevations, ranging from 0 to 1250 masl in Hajikend. By emphasizing the genetic variability of bread wheat within the framework of Azerbaijan's distinctive biodiversity, this research highlights the crucial role that diverse landscapes play in the conservation and sustainable use of plant genetic resources.

2 Material and methods

A total of 158 accessions of bread wheat (*Triticum aestivum* L.) were used as research materials. Among these, 35 are varieties developed over different years, while the remaining accessions were collected from various regions of the republic at different altitudes. These accessions were studied under field conditions for three years, selected based on their biomorphological traits, and their botanical variety was precisely identified.

Genomic DNA isolation was conducted following the CTAB DNA extracted protocol based on Doyle and Doyle [28]. Genotyping was performed using four SSR markers previously mapped to the A and B genomes (Table 1). The PCR conditions with fluorescent-dye labeled primers were as follows: initial denaturation at 95°C for 3 minutes; followed by 40 cycles of denaturation at 95°C for 1 minute, annealing at 50°C for 1 minute, and elongation at 72°C for 2 minutes; and a final elongation step at 72°C for 10 minutes. DNA fragments were analyzed on an ABI 3130xl Genetic Analyzer (Applied Biosystems/Thermo Fisher Scientific) [29]. Fragment analysis and allele calling were carried out using GeneMapper software version 3.7 (Applied Biosystems, Foster City, CA). Genetic diversity measures, including total number of alleles, expected heterozygosity (He), observed heterozygosity (Ho), and polymorphism information content (PIC), were calculated with PowerMarker version 3.51 [30]. Cluster analysis, Principal Coordinate analysis (PCoA), and the Unweighted Neighbor-Joining tree were generated using the DARwin 6.0 software package [31].

3 Results and discussion

Using SSR markers targeting the A and B genomes, genetic variation was investigated in a collection of bread wheat varieties and accessions from Azerbaijan. A total of 35 alleles were identified across 158 accessions using four SSR markers. The number of alleles per primer ranged from 4 to 16, with an average of 8.8 alleles. The highest number of alleles was detected with the *barc212* primer, while the lowest was noted with *barc117*. In a study by Salem et al., amplification of bread wheat accessions across 15 microsatellite loci resulted in an average of only 3.2 alleles per primer, and it was observed that the B genome exhibited greater polymorphism than the A and D genomes [32, 33]. In the present study, however, the A genome showed a higher number of alleles than the B genome, with mean allele number of 10 for the A genome and 7.5 for the B genome. A unique allele of 226 bp was identified with the *barc212* primer. Additionally, the Ugur variety displayed a rare allele combination (228/230) not observed in other genotypes, indicating heterozygosity at the *barc212* locus. Three unique alleles were identified with the *barc147* primer pair in genotypes 49, 128, and 144. No unique alleles were found with other primers, although some genotypes exhibited rare allelic combinations at the *barc117* and *barc218* loci.

The observed heterozygosity (Ho) within the collection ranged from 0.038 to 0.158, with an average of 0.084. The highest heterozygosity was recorded with the *barc182* primer (Table 1).

Expected heterozygosity (He), a key parameter of genetic diversity, ranged from 0.453 to 0.858, with the highest value observed for the *barc212* primer and the lowest for the *barc117* primer. The polymorphic information content (PIC) values ranged between 0.392 and 0.846. A statistically significant positive correlation was found between He, PIC values, and allele numbers at each locus ($r = 0.96$, $P < 0.01$). Despite the small number of primers, the average He and PIC for the collection were notably high. The average He and PIC for the 158 bread wheat varieties and accessions were 0.654 and 0.616, respectively.

Table 1. Genetic diversity parameters among the bread wheat genotypes

Primer	Locus	Allele number	Ho	He	PIC
barc212	2AS	16	0.057	0.858	0.846
barc147	3BS	10	0.082	0.711	0.671
barc117	5AS	4	0.038	0.453	0.392
barc182	7BL	5	0.158	0.593	0.555
Mean		8.8	0.084	0.654	0.616
Total		35			

Ho – observed heterozygosity, *He* – expected heterozygosity, *PIC* – polymorphism information content

Additionally, higher genetic diversity was detected in the collection with two of the four primers, barc212 and barc147, located on the short arms of chromosomes 2A and 3B, respectively. These findings are consistent with the results of Plaschke et al. who determined that a small number of markers were sufficient to differentiate closely related European wheat genotypes using SSR markers [34]. The results confirm the existence of a rich genetic resource in the microsatellite loci of the studied bread wheat collection, the majority of which are Azerbaijani accessions. The rich climatic and soil conditions of Azerbaijan, its long history of wheat cultivation, and decades of purposeful, successful breeding programs for bread wheat are the main contributors to this genetic wealth. Dvorak et al. identified the southwestern and southeastern regions of the Caspian Sea as one of the centers of origin and domestication of *T. aestivum*, noting the presence of significant genetic diversity in the species [35].

Bread wheat is known to have lower polymorphism compared to cereal crops like rice, maize, or barley, and this level of polymorphism is unevenly distributed throughout the hexaploid genome. Among the genomes, the D genome is relatively conserved, making gene mapping in this genome more challenging [27]. In contrast, the A and B genomes exhibit greater variation, and there is high transferability (>70%) of SSR markers specific to the A/B genomes to D genome species. Although the number of primers used in the current study is small, the high genetic diversity observed may be attributed to the fact that the markers were specific to the A/B genomes.

The accessions used in the study belong to 25 different botanical varieties, some of which are represented by a very small number of accessions. Comparative analysis of expected heterozygosity (He) and polymorphic information content (PIC) across botanical varieties represented by five or more accessions revealed that these genetic diversity parameters do not depend on the number of accessions (Table 2). The obtained results are consistent with findings from GBS and amplicon sequencing. For instance, the highest genetic variation across 4 microsatellite loci was observed in just 7 accessions of the var. *ferrugineum* botanical variety (He = 0.661; PIC = 0.616). This was followed by var. *alborubrum* (He and PIC for 41 accessions were 0.630 and 0.582, respectively), and then var. *barbarossa* (He = 0.615) with 17 accessions, and var. *erythrosperrum* (He = 0.614), all exhibiting similar diversity indicators. The least diversity was recorded for var. *albidum* botanical variety (He = 0.220; PIC = 0.202).

Table 2. Genetic parametrs obtained using SSR markers for various botanical varieties of bread wheat

Botanical varieties	Accession number	Ho	He	PIC
var. <i>albidum</i>	5	0.150	0.220	0.202
var. <i>alborubrum</i>	41	0.049	0.630	0.582
var. <i>barbarossa</i>	5	0.050	0.615	0.565
var. <i>erythroleucon</i>	5	0.050	0.545	0.444
var. <i>erythrosperrum</i>	17	0.147	0.614	0.573
var. <i>ferrugineum</i>	7	0.107	0.661	0.616
var. <i>graecum</i>	12	0.021	0.556	0.527
var. <i>hostianum</i>	7	0.071	0.510	0.469
var. <i>lutescens</i>	16	0.094	0.607	0.567
var. <i>milturum</i>	15	0.017	0.587	0.550

Ho – observed heterozygosity, *He* – expected heterozygosity, *PIC* – polymorphism information content

Based on the SSR data, Nei's genetic distance index between the accessions was calculated, and a dendrogram reflecting their genetic relationship was constructed (Fig. 1). The genetic distance index between genotypes ranged from 0 to 1, with an average of 0.623 units. This suggests that most of the studied bread wheat genotypes are genetically distinct and distant from one another. The dendrogram revealed the grouping of the accessions into three main clusters, two of which contained numerous small sub-clusters and groups. Notably, the Murov 2 variety formed an independent cluster (Cluster III), indicating that this variety is genetically distinct and unique compared to others, based on the studied microsatellite loci. It is important to note that the Murov 2 wheat variety, developed from the cross between the US variety and the Russian variety Spartak, exhibited a unique allele combination (116/131) at the *barc182* microsatellite locus.

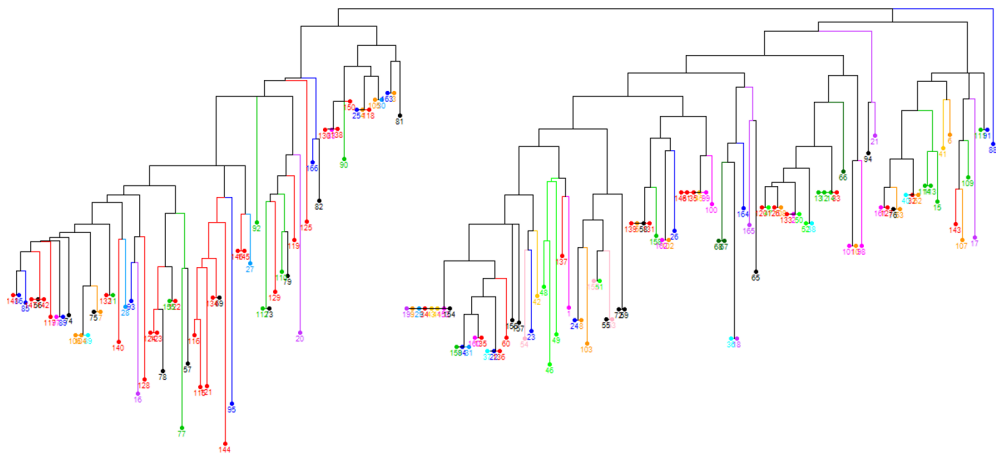


Fig. 1. Dendrogram illustrating the genetic relationships among 158 *T. aestivum* accessions based on the Nei genetic distance index. Different colors represent distinct botanical varieties.

Cluster I was divided into two subclusters comprising 63 genotypes, and many of the smaller groups that emerged from further branching showed genetic identity among botanical varieties and accessions. This cluster was dominated by genotypes of Var. *alborubrum*.

Cluster II included 59% of the accessions, with most genotypes showing a high degree of similarity to each other. In general, clustering of genotypes from the same botanical varieties or geographic regions was not observed in the dendrogram. Among the studied cultivars, genetic identity was recorded between Grekum 75/50 and Arzu, as well as between Guneshli and Saba (Guneshli) at the analyzed loci.

Three accessions collected in Hajikend at an altitude of 1258 m above sea level were grouped into different clusters with genetic distances ranging from 0.33 to 0.5. This change may be related to their classification as separate botanical varieties. Similarly, Kakhendi accessions collected at 700–800 m above sea level were distributed into different clusters with a genetic distance of 0.67. Of the five accessions collected in Nakhchivan at mid-altitudes (500–800 m above sea level), four, all classified as var. *alborubrum*, were grouped into the first cluster. In contrast, one genotype from var. *erythrosperrum* was placed into a separate third cluster. Plants at high altitudes undergo genetically adaptive evolution that increases their survival in harsh conditions characterized by prolonged exposure to elevated UVB radiation, low temperatures, and hypoxia [12]. Principal coordinate analysis (PCoA) was performed to assess the distribution of bread wheat genotypes in the scatter plot, with the first two coordinates explaining 32.7% of the variation in the collection (Fig. 2).

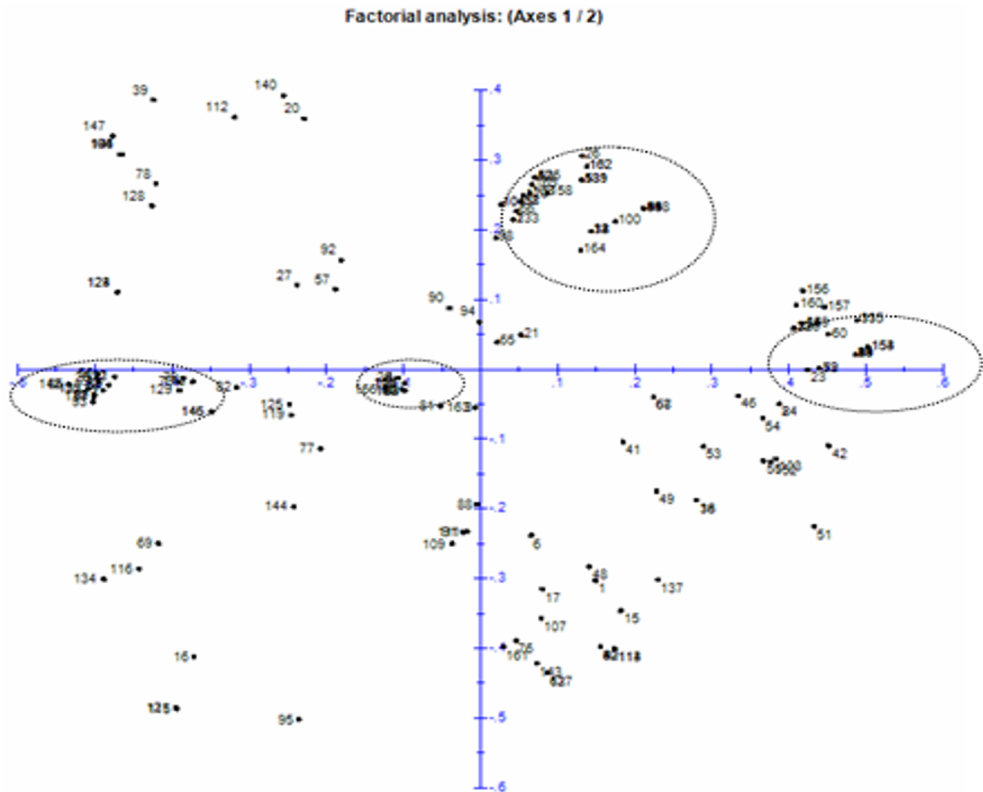


Fig. 2. PCoA analysis illustrating the clustering of *T. aestivum* accessions on a scattered plot. A group of genotypes that are genetically very similar is highlighted within a circle.

Compared to cluster analysis, PCoA described the genetic relationships between samples more clearly and allowed us to distinguish between groups that are either very similar (or identical) on the coordinate plane or, conversely, genetically distant from each

other. For example, the upper right quadrant consists of two groups of genotypes that are genetically very close, while the upper left and lower right quadrants contain samples with significant genetic distances between them. Another group of genetically identical genotypes is located along the coordinate axis in the lower left quadrant, while other samples in this quadrant are more scattered and are characterized by the highest values of the genetic distance index. Species var. *lutescens* and var. *alborubrum* were the most genetically similar (0.0623), while var. *leucospermum* and var. *pyrotrix* showed the highest genetic distance (0.679).

In the dendrogram reflecting the genetic relationships between the botanical varieties (Fig. 3), four clusters were identified. Cluster I is divided into two subclusters, where var. *lutescens*, var. *alborubrum*, and var. *erythrosperrum* are closely related. The second subcluster contains var. *graecum* and var. *ferrugineum* with a genetic distance between them of 0.156. Cluster II, consisting of three botanical varieties, shows a relatively high genetic distance between var. *glaucolutescens* and var. *pyrotrix* (0.488). Cluster III includes var. *albidum* and var. *erythroleucon* (GMI = 0.219), while cluster IV consisted of four botanical varieties. The genetic distance between var. *barbarossa* and var. *leucospermum* was 0.118, indicating close genetic similarity due to common alleles.

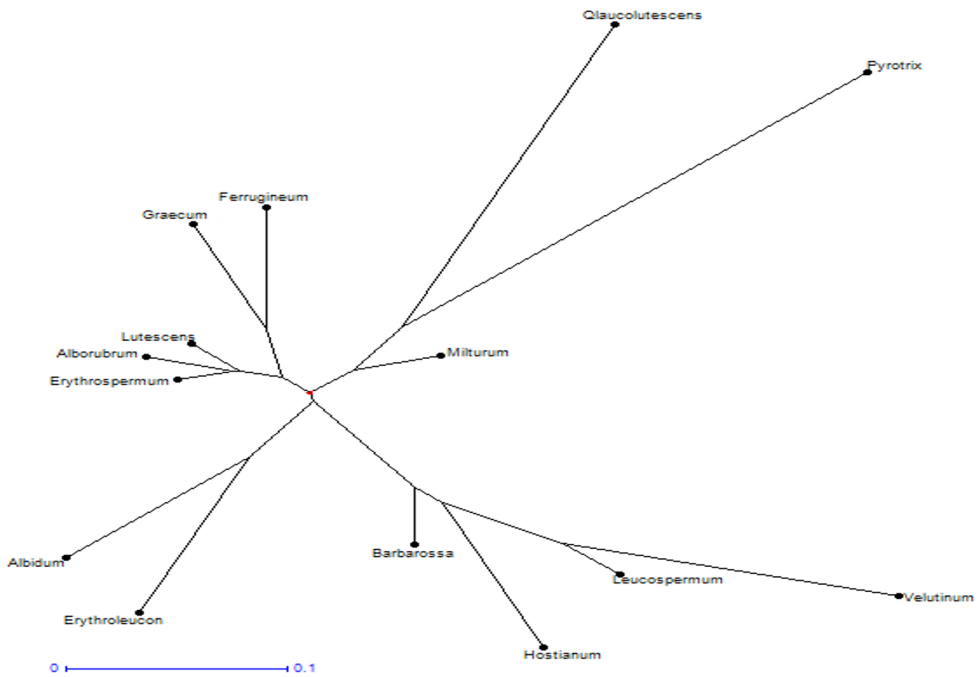


Fig. 3. Dendrogram illustrating the genetic relatedness among *T. aestivum* botanical varieties based on the Nei genetic distance index.

4 Conclusions

A study of a local bread wheat collection using SSR marker technology revealed significant variability and polymorphism in DNA microsatellite regions among the wheat accessions. Despite the limited number of primers, the collection was found to be genetically diverse.

Accessions from higher altitudes such as Hajikend (1258 m a.s.l.), Khankendi (700-800 m a.s.l.), Nakhchivan (500-860 m a.s.l.) and others contributed significantly to this diversity as they were genetically distinct and fell into different clusters. These results provide valuable information for the selection of parental combinations with distinct genetic profiles in bread wheat breeding programs and for their use in hybridization to generate a wide range of recombinants. Furthermore, the results of this study can inform conservation strategies and contribute to the protection of these vital ecosystems, including high mountain landscapes, while enhancing food security by increasing crop resilience.

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