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# Exploring the genetic diversity and population structure of Serbian and selected European bread wheat cultivars through iPBS-retrotransposon markers

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**Abstract:** Bread wheat is a globally vital crop, sustaining millions and contributing to food security. The present study investigated the molecular characterization of 60 bread wheat accessions, using 12 interprimer binding site (iPBS) retrotransposon markers, which yielded a total of 260 distinct bands. Out of the 260 bands, 42 were monomorphic while the remaining 218 (83.84%) were polymorphic, with polymorphism information content values ranging from 0.38 to 0.45. Genetic diversity indices, including Shannon's information index (I = 0.01–0.53), effective number of alleles (Ne = 1.00–1.63), gene diversity (He = 0.0037–0.36), and marker index (MI = 0.31–0.51), revealed moderate variability across the accessions. Analysis of molecular variance indicated 99% genetic variation within populations, underscoring the genetic richness of the germplasm. Principal coordinate analysis, neighbor-joining tree, and model-based STRUCTURE clustering divided the accession into two distinct groups. The average genetic distance was 0.23, with a minimum of 0.063 between Mohikana and NS Lenija and a maximum of 0.56 between LG Airbus and BC Bernarda accessions. Given their high genetic divergence, LG Airbus and BC Bernarda are suggested as potential candidate parents for future wheat breeding programs. Our study highlights the genetic variation within Serbian wheat germplasm and could be valuable for parental selection and the strategic planning of future breeding programs.

**Key words:** Genetic diversity, iPBS-retrotransposon markers, molecular characterization, Shannon's information index, sustainable agriculture

## 1. Introduction

Wheat (*Triticum* spp.), belonging to the family *Poaceae*, serves as an integral staple grain with immense global significance, undergirding food security and sustaining millions of people worldwide (Baloch et al., 2024; Chen et al., 2024). Wheat cultivation occupies a massive area of 219 million hectares, producing an annual yield of approximately 760 million tons (Draz et al., 2024). Wheat, an allohexaploid cereal (2n = 6x = 42, AABBDD genome), originated from two polyploidization events approximately 0.5 million years ago involving *Aegilops speltoides* and *Triticum urartu* related species (Marcussen et al., 2014; Nadeem, 2021).

The continuous practice of primitive farming and generational selection by farmers resulted in wheat populations uniquely suited to regional environments. As trade networks expanded, these native wheat ecotypes, once unnamed, became associated with the regions where they were cultivated. It was not until the mid-19th century that significant efforts to improve wheat production and selection were made. The rediscovery of Mendelian genetics in the early 20th century marked a breakthrough that profoundly impacted plant breeding practices worldwide, including in Serbia, which belonged to Yugoslavia during that period. Following World War 2, the rapid population

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expansion in Yugoslavia necessitated a significant increase in wheat production to meet the growing demand for food. To meet the escalating demand for wheat, the Yugoslav government imported San Pastore and 30 other varieties from Italy during the 1950s, with the objective of attaining self-sufficiency in wheat. These varieties were extensively cultivated and served as a foundation for plant breeders, who crossed them with domestic winter wheat varieties. The development of modern, high-yielding Yugoslav winter wheat cultivars can be divided into three stages. In the first stage, genes for winter hardiness were introduced into Italian semidwarf, early-maturing, high-yielding varieties through crosses with older domestic or West European cultivars. The second stage focused on introducing resistance to diseases like rust and powdery mildew. The third stage concentrated on improving bread-making quality, primarily through the use of the Russian cultivar Bezostaja1 (Jošt and Cox, 1989; Borojević and Borojević, 2005).

Climate change challenges global agriculture, requiring crops like wheat to adapt with greater resilience, productivity, and flexibility to unpredictable conditions (Altaf et al., 2024a; Šućur et al., 2025). Genetic diversity is crucial for improving crop plants like wheat, enabling the development of high yield, resistant varieties, and adaptability to diverse environmental conditions (Huang et al., 2002; Iqbal et al., 2023; Ceyhan et al., 2025). Understanding the properties of genetic resources and conducting targeted breeding experiments are essential to optimize the existing gene resources for wheat breeding. Modern plant breeding programs have led to a reduction in genetic diversity in wheat and other crops, as domestication and intensive selection methods have resulted in replaced diverse landraces with genetically distinct traits (Demirel et al., 2024). Molecular plant breeding expands useful genetic diversity for crop improvement (Ali et al., 2025; Mortazavi et al., 2025). Molecular markers offer a detailed view of wheat's genetic landscape, with each technique bringing unique advantages (Altaf et al., 2024b). Retrotransposons are highly mobile genetic elements that make up a significant portion of eukaryotic genomes, making them powerful tools for genetic diversity analysis (Kalender et al., 2010; Demirel et al., 2024). Multiple molecular marker techniques such as RAPD (Marić et al., 2004), RFLPs (Paull et al., 1998), AFLPs (Roy et al., 2004), DArT (Baloch et al., 2017), SCoT (Altaf et al., 2024c), EST (Chabane et al., 2007), STS (Chen et al., 1994), SNP (Ren et al., 2013), SSR (Huang et al., 2002), and ISSRs (Nazarzadeh et al., 2020) have been used to assess genetic diversity and associations among *Triticum* species. Kalendar et al. (2010) proposed the interprimer binding site (iPBS)-retrotransposon-based amplification technology as a flexible molecular tool that can overcome the limitations of traditional markers and provide valuable insights into genetic diversity and relationships across both the animal and plant kingdoms. iPBS-retrotransposon markers have shown remarkable potential in the investigation of genetic diversity and breeding of wheat. Recently, iPBS-retrotransposon markers have emerged as a versatile and essential tool for assessing genetic diversity across various plant species, including wheat (Demirel, 2020; Nadeem, 2021; Haliloğlu et al., 2023; Demirel et al., 2024; Baran, 2024). The present study used iPBS-retrotransposon markers to analyze the genetic diversity and population structure of Serbian and European bread wheat germplasm, providing insights for improved breeding and conservation programs.

## 2. Materials and methods

### 2.1. Plant material

The current investigation utilized 60 bread wheat cultivars as plant material (Table 1). These bread wheat cultivars, previously released by well-known institutes in Europe, were collected from the Faculty of Agriculture (UNSFA) and Institute of Field and Vegetable Crops, Novi Sad, Serbia, for the assessments of genetic diversity and molecular characterization. Out of the 60 bread wheat genotypes, 20 were Serbian bread wheat genotypes, while the rest of the plant material originated from following countries: France (25), Croatia (7), Italy (4), Mexico (2), Hungary (1), and Romania (1).

### 2.2. DNA isolation

Seeds of all collected germplasm were sown in a greenhouse at Sivas University of Science and Technology, Türkiye. Twenty days after sowing, the fresh green wheat leaves were used for DNA isolation. DNA extraction was performed using a standard cetyltrimethylammonium bromide (CTAB) protocol, as recommended by Diversity Arrays Technology (DArT) Pty Ltd. (Canberra, Australia), following the protocol of Doyle (1990). The quality and quantity of the extracted DNA were checked by 1% agarose gel and A260/A280 absorption in a NanoDrop instrument (Thermo Fisher Scientific, Waltham, MA, USA), respectively. A 5 ng/µL dilution was prepared from the stock DNA and used for PCR analysis.

### 2.3. Preparation of 5 ng/µL dilution from stock DNA

A ng/µL dilution refers to the concentration of a particular substance in a solution, measured in nanograms (ng) per microliter (µL). During the present study, the following steps were performed to prepare the 5 ng/µL dilution from the stock DNA. First, we determined the initial concentration (C1) in nanograms per microliter of the stock DNA. Next, we determined the initial volume (V1) in microliters. Then we determined the final volume (V2) in microliters. Next, we used the formula C1V1 = C2V2 to calculate the concentration of C2 to prepare the final dilution at 5 ng/µL.

**Table 1.** Plant material used during the present study.

| Sample No. | Genotypes | Year | Country | Institution |
|---|---|---|---|---|
| 1 | Falado | - | France 1 | Syngenta |
| 2 | Cellule | 2011 | France 2 | Florimond Desprez |
| 3 | KWS Marvel | 2019 | France 3 | KWS |
| 4 | Osmose | 2015 | France 4 | Caussade |
| 5 | Sonahine | 2020 | France 5 | Caussade |
| 6 | KWS Criterium | 1995 | France 6 | Hybritech |
| 7 | KWS Feria | 2009 | France 7 | KWS |
| 8 | Sofolk | 2014 | France 8 | Caussade |
| 9 | Solveig | 2011 | France 9 | Caussade |
| 10 | Solindo | 2016 | France 10 | Caussade |
| 11 | Centurion | 2014 | France 11 | Saaten Union |
| 12 | Sofru | 2009 | France 12 | Caussade |
| 13 | Sothys | 2014 | France 13 | Caussade |
| 14 | LG Aigle | 2012 | France 14 | LG |
| 15 | Sosthene | 2012 | France 15 | Caussade |
| 16 | Solenzara CS | 2014 | France 16 | Caussade |
| 17 | Providence | 2018 | France 17 | Florimond Desprez |
| 18 | LG Airbus | 2014 | France 18 | LG |
| 19 | Presnatce | 2020 | France 19 | Florimond Desprez |
| 20 | LG Anapurna | 2013 | France 20 | LG |
| 21 | Nogal | 2010 | France 21 | Florimond Desprez |
| 22 | Alhambra | 2010 | France 22 | LG |
| 23 | LG Alcantara | 2013 | France 23 | LG |
| 24 | Winner | 2018 | France 24 | Florimond Desprez |
| 25 | KWS Modern | 2012 | France 25 | KWS |
| 26 | BC Lorena | 2011 | Croatia 1 | Bc Institute for breeding and seed production of field crops Zagreb |
| 27 | Renan | 2012 | Croatia 2 | GRI OBTENTIONS |
| 28 | BC Bernarda | 2012 | Croatia 3 | Bc Institute for breeding and seed production of field crops Zagreb |
| 29 | BC Anica | 2009 | Croatia 4 | Bc Institute for breeding and seed production of field crops Zagreb |
| 30 | BC Darija | 2011 | Croatia 5 | Bc Institute for breeding and seed production of field crops Zagreb |
| 31 | BC Opsesija | 2015 | Croatia 6 | Bc Institute for breeding and seed production of field crops Zagreb |
| 32 | BC Ljepotica | 2015 | Croatia 7 | Bc Institute for breeding and seed production of field crops Zagreb |
| 33 | Katou | 2014 | Italy 1 | Apsovsementi |
| 34 | Apsov Katon | 2014 | Italy 2 | Apsovsementi |
| 35 | Marinello | 2008 | Italy 3 | KWS Momont |

**Table 1.** (Continued).

| | | | | |
|---|---|---|---|---|
| 36 | Algeri | 2020 | Italy 4 | Apsovsementi |
| 37 | Eswyt 50 | 1992 | Mexico 1 | CIMMYT Line |
| 38 | Sawyt 47 | 1992 | Mexico 2 | CIMMYT Line |
| 39 | BG Converta | 2020 | Serbia 1 | Biogranum |
| 40 | Quattrona | 2021 | Serbia 2 | AgroSava |
| 41 | BG Flexa | 2020 | Serbia 3 | Biogranum |
| 42 | NS Igra | | Serbia 4 | Institute of Field and Vegetable Crops, Novi Sad |
| 43 | NS Modena | | Serbia 5 | Institute of Field and Vegetable Crops, Novi Sad |
| 44 | Nataša | 2003 | Serbia 6 | Institute of Field and Vegetable Crops, Novi Sad |
| 45 | Mohikana (line) | | Serbia 7 | Line, still not recognized |
| 46 | NS Lenija | | Serbia 8 | Institute of Field and Vegetable Crops, Novi Sad |
| 47 | Simonida | 2003 | Serbia 9 | Institute of Field and Vegetable Crops, Novi Sad |
| 48 | NS Epoha | | Serbia 10 | Institute of Field and Vegetable Crops, Novi Sad |
| 49 | NS Grivna | | Serbia 11 | Institute of Field and Vegetable Crops, Novi Sad |
| 50 | PKB Pahuljica | | Serbia 12 | PKB Agroeconomic Institute |
| 51 | Zvezdana | 2006 | Serbia 13 | Institute of Field and Vegetable Crops, Novi Sad |
| 52 | PKB Ratarica | | Serbia 14 | PKB Agroeconomic Institute |
| 53 | PKB Talas | | Serbia 15 | PKB Agroeconomic Institute |
| 54 | BG Klimatika | 2020 | Serbia 16 | Biogranum |
| 55 | BG Ikona | 2019 | Serbia 17 | Biogranum |
| 56 | BG Logika | 2020 | Serbia 18 | Biogranum |
| 57 | Bisenija | 2021 | Serbia 19 | Agrosava |
| 58 | BG Elastika | 2020 | Serbia 20 | Bigranum |
| 59 | GK Koros | 2010 | Hungary 1 | GK |
| 60 | Amicus | 2016 | Romania 1 | Saatzucht Donau |

**2.4. PCR amplification using iPBS primers**

During the preliminary phase of the study, eight DNA samples were randomly chosen to select polymorphic markers that would be further used in the genetic diversity analysis. The selected samples were evaluated using iPBS primers, where 12 out of 30 highly polymorphic primers were selected for the next phase of the study (Table 2). The PCR product contained 2.5 µL (5 ng/µL) of DNA, 1 µL of 10x PCR buffer (+KCL and –$MgCl_2$), 1 µL of 0.2 mM dNTPs, 1.5 of 0.5 µM of each iPBS primer, 0.15 µL of Taq DNA polymerase (DreamTaq, Thermo Scientific), and 1 µL of $MgCl_2$ and 2.85 µL of $dH_2O$ was added to make the final volume of 10 µL of the reaction mixture. The PCR conditions included an initial denaturation at 95 °C for 5 min followed by 35 cycles of amplification with denaturation at 95 °C for 1 min, annealing at 50–52 °C for 1 min (specific for each primer pair), and the final extension step of 72 °C for 8 min. The amplified PCR products were analyzed by 2.5% agarose gel electrophoresis for 2 h and 30 min at 115 V. Following ethidium bromide staining, the PCR products were visualized under UV light using a Gel Documentation System (Bio-Rad, Hercules, CA, USA). A 100-bp DNA marker (ranging from 100 to 3000 bp; LOT 2674431, Invitrogen, Carlsbad, CA, USA) was used to estimate the PCR product size.

**2.5. Statistical analysis**

After the visualization of the PCR products, a binary score model was applied for scoring. According to this model, a value of 0 signifies the absence of a band, while a value of 1 indicates its presence. Furthermore, we calculated

**Table 2.** List of iPBS markers used during the current study.

| Primer | Sequence | Annealing temperature |
|---|---|---|
| iPBS-2244 | GGAAGGCTCTGATTACCA | 50 °C |
| iPBS-2246 | ACTAGGCTCTGTATACCA | 50 °C |
| iPBS-2252 | TCATGGCTCATGATACCA | 52 °C |
| iPBS-2388 | TTGGAAGACCCA | 50 °C |
| iPBS-2385 | CCATTGGGTCCA | 50 °C |
| iPBS-2375 | TCGCATCAACCA | 52 °C |
| iPBS-2386 | CTGATCAACCCA | 50 °C |
| iPBS-2278 | GCTCATGATACCA | 50 °C |
| iPBS-2231 | ACTTGGATGCTGATACCA | 52 °C |
| iPBS-2257 | CTCTCAATGAAAGCACCA | 50 °C |
| iPBS-2087 | GCAATGGAACCA | 52 °C |
| iPBS-2380 | CAACCTGATCCA | 50 °C |

certain parameters for genetic diversity determination, including Shannon's information index (I), gene diversity (He), Nei's genetic distance, and effective number of alleles (Ne). All these measures were determined using the software POPGENE version 1.32 (Yeh et al., 1997), and the polymorphism information content (PIC) values were calculated using Roldán-Ruiz et al.'s (2000) methodology. Both the principal coordinate analysis (PCoA) and the analysis of molecular variance (AMOVA) were performed using the software GenAlEx version 6.5 (Peakall and Smouse, 2012). In order to determine the population structure of 60 different bread wheat genotypes, the software STRUCTURE (Pritchard et al., 2000) was used. Additionally, neighbor-joining analysis was carried out with the statistical software R in order to explore the degree of genetic similarity between the individual genotypes. Evanno et al. (2005) provided a framework that was followed in order to determine the ideal number of clusters, which corresponds to K subpopulations. The values of the clusters were evaluated in a range from 1 to 10. Each study was carried out with a burn-in period and a Markov chain Monte Carlo (MCMC) algorithm with a value of 50,000. Additionally, the number of iterations was kept at 10. This was followed by the processing of the results with STRUCTURE HARVESTER version 0.99 (Earl and vonHoldt, 2012) in order to ascertain the K value that was the most suitable. The ΔK values, which indicated the optimal clustering solution, were visualized using the R package "POPHELPER" (Francis, 2017).

## 3. Results

### 3.1. Polymorphism revealed by iPBS markers

Twelve highly polymorphic iPBS primers were employed in the diversity analysis of the examined bread wheat germplasm. A total of 260 amplified bands were detected in the 60 bread wheat accessions (Table 3). Out of the 260 bands, 42 were monomorphic, whereas 218 were polymorphic. The mean number of obtained bands per primer was 21.66, with an average of 18.16 polymorphic bands per primer. iPBS-2375, iPBS-2385, and iPBS-2388 produced the highest numbers of bands: 35, 29, and 28, respectively. Thus, the marker iPBS-2087 demonstrated the highest polymorphism rate at 100%, whereas iPBS-2385 displayed a polymorphism rate of 96.55% (Table 3). The lowest number of polymorphisms was seen in iPBS-2246, yielding 10 out of 15 bands with an average of 66.66%. PIC values, which reflect marker polymorphism, varied from 0.38 to 0.45 for iPBS (2385, 2386, 2257, and 2087), and iPBS-2380 16, respectively, yielding an overall average PIC value of 0.40 (Table 3). The highest Ne was recorded was 1.63 for iPBS-2385, followed by 1.54 for iPBS-2375, while the lowest Ne of 1.00 was noted for iPBS-2231. The mean Ne of 1.29 was documented for all employed iPBS markers. The maximum and least Shannon's information index (I) reported were 0.53 for iPBS-2385 and 0.01 for iPBS-2231, respectively, with an average I of 0.26 for all iPBS markers. The maximum He detected was 0.36 with iPBS-2385, while the minimum was 0.0037 with iPBS-2386 and iPBS-2380. The mean gene diversity value for all employed

**Table 3.** Results of genetic diversity parameters using the iPBS markers system.

| Primer | Total bands | PB | PB % | PIC | Marker index | Ne | He | I |
|---|---|---|---|---|---|---|---|---|
| iPBS-2244 | 24 | 19 | 79.16 | 0.41 | 0.42 | 1.35 | 0.22 | 0.35 |
| iPBS-2246 | 15 | 10 | 66.66 | 0.4 | 0.51 | 1.39 | 0.23 | 0.34 |
| iPBS-2252 | 23 | 20 | 86.95 | 0.39 | 0.46 | 1.44 | 0.27 | 0.41 |
| iPBS-2388 | 28 | 23 | 82.14 | 0.41 | 0.42 | 1.44 | 0.26 | 0.39 |
| iPBS-2385 | 29 | 28 | 96.55 | 0.38 | 0.49 | 1.63 | 0.36 | 0.53 |
| iPBS-2375 | 35 | 29 | 82.85 | 0.41 | 0.43 | 1.54 | 0.3 | 0.44 |
| iPBS-2386 | 18 | 12 | 66.66 | 0.39 | 0.47 | 1.01 | 0.01 | 0.03 |
| iPBS-2278 | 17 | 14 | 82.35 | 0.38 | 0.48 | 1.02 | 0.02 | 0.04 |
| iPBS-2380 | 15 | 14 | 93.33 | 0.45 | 0.31 | 1.01 | 0.01 | 0.02 |
| iPBS-2231 | 18 | 14 | 77.77 | 0.41 | 0.46 | 1.00 | 0.02 | 0.01 |
| iPBS-2257 | 20 | 17 | 85 | 0.38 | 0.49 | 1.12 | 0.07 | 0.12 |
| iPBS-2087 | 18 | 18 | 100 | 0.38 | 0.48 | 1.49 | 0.29 | 0.44 |
|  | 260 | 218 | 92.5 | 0.40 | 0.45 | 1.29 | 0.17 | 0.26 |

iPBS primers was 0.17. The highest marker index of 0.51 was observed for iPBS-2246, while the lowest index of 0.31 was observed for iPBS 2380. The recorded average marker index for all primers was 0.45. The assessment of the final outcomes revealed a mean genetic distance of 0.23, with a minimum genetic distance of 0.063 between Mohikana (line) and NS Lenija, and a maximum genetic distance of 0.56 between LG Airbus and BC Bernarda.

The model-based STRUCTURE algorithm grouped the 60 wheat accessions into two populations (population 1 and population 2) based on their collection regions, while 11 accessions remained unclassified (Figure 1). Furthermore, population 1 included 30 bread wheat accessions (France 16, France 17, France 18, Mexico 1, Croatia 2, Hungary 1, Serbia 2, France 24, France 5, France 6, France 7, Italy 3, France 21, Romania 1, France 11, Croatia 1, Serbia 1, France 19, France 13, Serbia 8, Serbia 11, Serbia 9, Serbia 13, Serbia 6, Serbia 3, Serbia 5, Serbia 7, Croatia 5, Serbia 12, and Serbia 4); on the other hand, 19 bread wheat accessions (Italy 1, Croatia 4, Croatia 3, Serbia 20, Mexico 2, Serbia 16, France 3, Serbia 19, France 25, Italy 2, Serbia 15, France 23, Croatia 6, Serbia 18, France 8, France 2, France 1, France 4, and Serbia 10), were present in population 2, with 11 present as an unclassified population.

The neighbor-joining assessments categorized the entire wheat germplasm into two groups (A and B) based on their collecting locations (Figure 2). Group B was further subdivided into two groups, B1 and B2. The AMOVA results indicated that 99% of genetic variations exist within the assessed population, in contrast to 1% among the populations (Table 4). PCoA enhances the clustering of the model-based structural method by categorizing the 60 gathered bread wheat accessions into two populations. The results clearly indicate that population A exhibits greater genetic variety than population B (Figure 3). Furthermore, during the population structure analysis, the delta K plot revealed a peak at K = 2, indicating that two clusters best represent the genetic structure in our dataset (Figure 4).

## 4. Discussion

Genetic diversity and population structure in wheat are vital for ensuring crop productivity and sustainability. iPBS markers play a key role in detecting genetic polymorphism, identifying unique traits, and tracing the evolutionary history of crops (Tajibayev et al., 2023). These markers are cost-effective, reproducible, and straightforward, facilitating the study of population structure, breeding strategies, and marker–trait associations (Nadeem, 2021; Güngör et al., 2022). They are also instrumental in

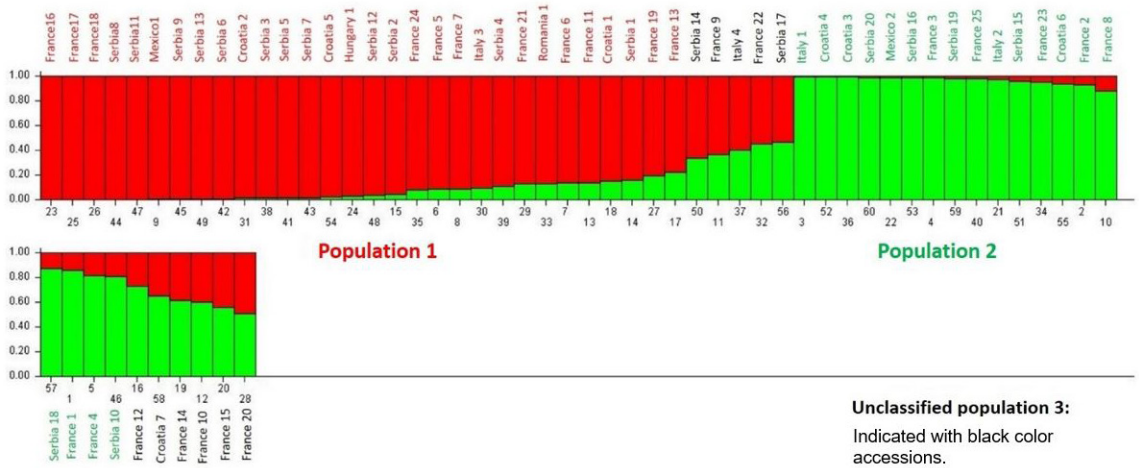


**Figure 1.** The structure-based clustering of 60 bread wheat accession using iPBS markers.

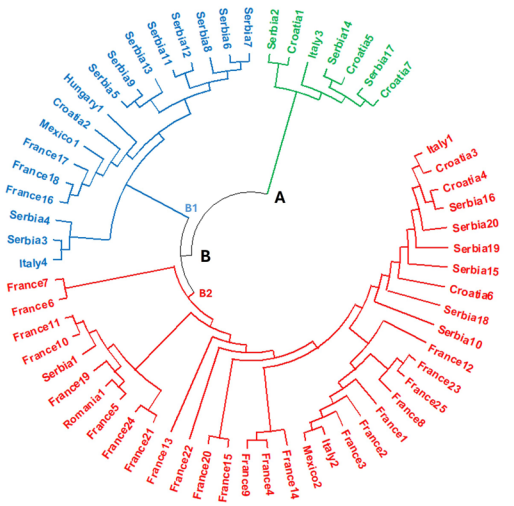


**Figure 2.** The study utilized iPBS markers for neighbor joining-based clustering among the studied bread wheat germplasm.

**Table 4.** AMOVA within and among populations of bread wheat germplasm using iPBS markers.

| Source | df | SS | MS | Est. Var. | Perc. (%) |
|---|---|---|---|---|---|
| Among populations | 4 | 15.510 | 3.877 | 0.030 | 1% |
| Within populations | 53 | 189.757 | 3.580 | 3.580 | 99% |
| Total | 57 | 205.266 | | 3.611 | 100% |

df: degree of freedom; MS: mean squares; SS: sum of squares; Perc. (%): percentage; Est. Var: estimated variance.

identifying genetic bottlenecks, monitoring gene flow, and detecting introgression events, thereby enhancing wheat's adaptability to environmental changes. The versatility of iPBS markers, particularly in polyploid species like wheat, allows for the discovery of novel alleles, contributing to improved food security (Haliloğlu et al., 2023). The present investigation utilized a total of 12 iPBS markers to assess the genetic diversity across 60 bread wheat accession and revealed a total of 260 bands, while 218 (83.84%) were highly polymorphic. All the applied primers exhibited good polymorphism rates and proved that they are good in assessing the genetic diversity of wheat plants. The

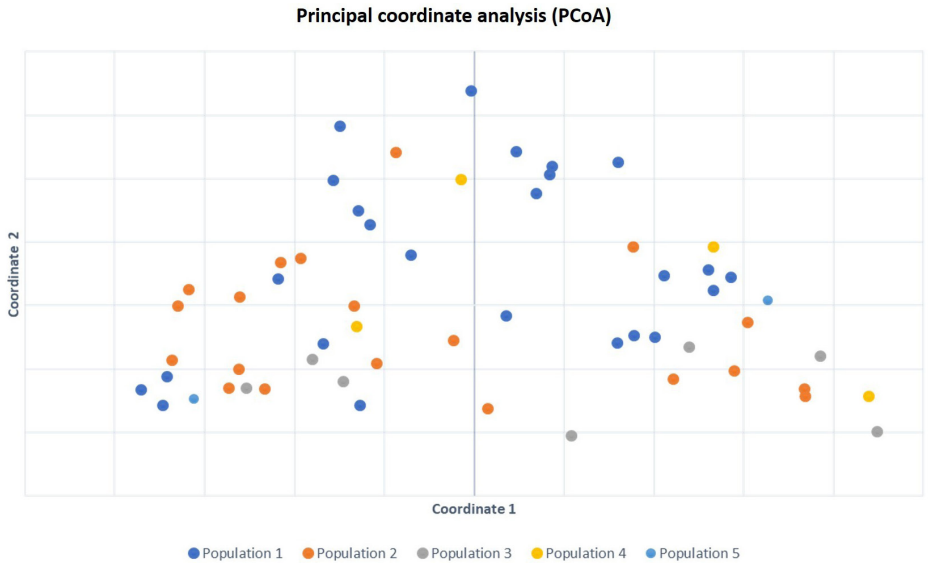


**Figure 3.** Principal coordinates analysis of bread wheat cultivars using the iPBS marker.

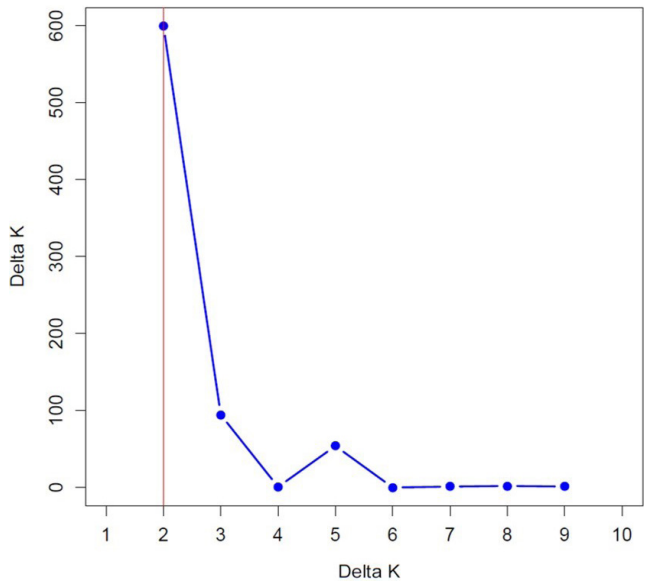


**Figure 4.** Delta K value indicating the existence of two populations of bread wheat germplasm.

results of our study were better compared to published studies on wheat and other important agricultural crops. Nadeem (2021) conducted a study to assess the genetic diversity and molecular characterization of 74 Turkish bread wheat accessions using 13 highly polymorphic iPBS markers and yielded a total of 152 bands, of which 111 are polymorphic (73.02%). Baloch et al. (2015) conducted a study to assess the genetic diversity of 138 peas accessions using a 12 highly polymorphic iPBS markers and yielded a total of 106 bands, of which 81 are polymorphic (76.41%). In another study, Arystanbekkyzy et al. (2019) performed a study to assess the genetic diversity of Turkish *Triticum turgidum* subsp. *dicoccoides* wheat germplasm using nine iPBS markers and yielded a total of 171 with 140 polymorphism (81.83%) bands. Milovanov et al. (2019) assessed the genetic diversity of grapevine germplasm using iPBS markers. The results of their study revealed a total of 1412 bands with 618 (43.76%) polymorphism bands. Furthermore, the obtained polymorphism rate (83.84%) during the currently undertaken study is lower than those reported in other studies utilizing distinct marker systems (Demirel, 2020; Eren et al., 2023; Haliloğlu et al., 2023; Demirel et al., 2024). The PIC values, reflecting marker polymorphism, ranged from 0.38 to 0.45, with an overall

average PIC value of 0.40 recorded during the current investigation. The obtained PIC value is higher than those in previously reported studies; for example, Demirel et al. (2024) found 0.22 in Turkish wheat germplasm, Kizilgeci et al. (2022) found 0.35 in an *Aegilops* species, Demirel (2020) found 0.19 in emmer wheat genotypes, Khaled et al. (2015) found 0.10–0.15 in wheat germplasm, Nadeem (2021) found 0.38 in Turkish bread wheat germplasm, and Kumar et al. (2020) found 0.35 in bread wheat germplasm. Variations in PIC values may be due to different marker systems or genotypes, but they still serve as a valuable indicator of marker informativeness in capturing genetic diversity in wheat germplasm.

In the current study, the maximum number of effective alleles (Ne) of 1.63 was observed with iPBS-2385, followed by 1.54 with iPBS-2375, and the minimum Ne of 1.00 was recorded with iPBS-2231. The average Ne of 1.29 was recorded for all utilized iPBS markers. This average Ne value is higher than what Nadeem (2021) found in wheat (1.24), Eren et al. (2023) found in alfalfa (1.26), and Haliloğlu et al. (2023) found in wheat germplasm (1.15). The Ne value obtained in the current study is lower than those found by Demirel (2020) and Demirel et al. (2024). Additionally, the He value (0.17) obtained in our study is lower than those in reported studies using iPBS markers (Demirel, 2020; Nadeem, 2021; Demirel et al., 2024). Moreover, Shannon's information index (I) for all iPBS markers was recorded as 0.26 during the present study and that surpassed those reported by Nadeem (2021) in wheat utilizing iPBS markers for wheat germplasm characterization. The maximum marker index 0.51 was recorded for iPBS-2246 and the minimum 0.31 was recorded for iPBS-2380. Our research reveals increased diversity indices in wheat germplasm due to variations in germplasm and the iPBS-retrotransposons marker system's exceptional reproducibility and worldwide applicability, making it a priority for molecular characterization of wheat germplasm.

Wheat plant population structure was analyzed using iPBS-retrotransposons markers. These markers reveal genetic differentiation across populations, aiding in understanding adaptation, breeding potential, and conservation strategies (Nadeem et al., 2018). They also help identify unique genetic lineages and provide insights into evolutionary relationships. iPBS-based studies help design breeding programs and understand gene flow and domestication effects on wheat genetic diversity. However, the structure clustering analysis divided all 60 bread wheat accessions into two distinct groups: population 1 and population 2 (Figure 1). Population 1 mainly comprised accessions from France and Serbia. This clustering can be explained by the fact that French wheat breeding material has been widely used in Serbian breeding programs. Additionally, most European wheat varieties share common ancestral lines, which were introduced into Europe during the Green Revolution. The modern bread wheat varieties that emerged from this period were shaped by the introduction of the Norin 10 dwarfing gene from Japanese wheat, which played a key role in enhancing wheat yield and overall robustness (Šućur et al., 2024). However, while Japanese wheat did not directly contribute as a progenitor to European bread wheat, the dwarfing gene it introduced was crucial in shaping contemporary wheat varieties. Population 2, on the other hand, consisted of an admixture of accessions from various countries, although French and Serbian genotypes were still dominant, likely because these were the majority of the samples in our study. Furthermore, wheat from Croatia and Italy, countries geographically close to Serbia, exhibit genetic similarities with Serbian wheat, which likely contributes to the observed clustering. This division and classification of these genotypes is evidence of wheat plant genetic diversity, so the assessed germplasm is highly recommended for breeding programs. Neighbor-joining analysis results separated the whole bread wheat germplasm into two groups (A and B) (Figure 2). Group B is further subdivided into B1 and B2 subgroups. However, the results of the neighbor-joining analysis were unclear and inconsistent. The division of the collected germplasm is not well understood. Therefore, the present study validated the results of previous studies by using iPBS and other genetic markers in crop genetic diversity (Nadeem, 2021; Smirnova et al., 2022; Altaf et al., 2024c; Yalinkiliç et al., 2024), and we also reported more structured analysis results more accurately than the neighbor-joining analysis results.

AMOVA is a statistical method used to partition genetic variation within and among populations. Using iPBS markers, researchers can identify genetic variation, within-population diversity, and among-population differences, aiding in understanding wheat germplasm, identifying unique resources, and guiding breeding strategies (Sameeullah et al., 2025; Yeşil Bayrıl et al., 2024). The AMOVA analysis revealed that 99% of the total genetic variation was attributable to differences within the assessed population, while only 1% was due to variation among populations (Table 4). PCoA is a statistical technique used in wheat plant genetic diversity analysis to visualize genetic relationships among genotypes. It uses a distance matrix to project genotypes into coordinate space, identifying genetic clusters and diversity patterns (Coşkun, 2023). PCoA helps breeders identify genetically distinct accessions for trait improvement and selection of parental lines for hybridization, ultimately improving wheat yield, quality, and stress tolerance. The PCoA algorithm's clustering was enhanced by dividing the 60 bread wheat accessions into two populations, revealing that population A exhibited greater genetic diversity (Figure 3).

Furthermore, during the population structure analysis, the delta K plot revealed a peak at K = 2, indicating that two clusters best represent the genetic structure in our dataset (Figure 4).

## 5. Conclusion

Our study shows the effectiveness of iPBS-retrotransposon markers in revealing genetic diversity and population structure among Serbian and European bread wheat germplasm. A high polymorphism rate (83.84%) among the 60 accessions was observed, with significant genetic diversity indices, such as a mean genetic distance of 0.23 and a Shannon's information index (I) of 0.26. AMOVA revealed the genetic variation within populations (99% of total variation), highlighting the germplasm's richness, with markers like iPBS-2385 showing the highest polymorphism rate (96.55%) and effective alleles (1.63). Population structure analysis clustered the accessions into two distinct groups, reflecting shared ancestry and breeding history, particularly between Serbian and French cultivars, with population A demonstrating greater genetic diversity. Notably, accessions like LG Airbus and BC Bernarda, with the highest genetic distances, were identified as promising candidates for breeding programs focused on improving drought tolerance, disease resistance, and yield. These findings validate the utility of iPBS markers for comprehensive genetic characterization and provide critical insights for future breeding and conservation strategies to meet global agricultural challenges.

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## Contribution of authors

Methodology: RŠ, AA, PM, MTA, YSC and MT; Validation-Formal analysis: MAN; Investigation: VM and FSB; Data curation: AA, MTA and BJ; Writing—original draft preparation: RŠ, YSC, AA, PM, and MTA; Review and editing: SF, YSC, FSB, and MAN; Supervision: FSB, and VM. All authors have read and agreed to the published version of the manuscript.

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## Conflict of interest

The authors declare that they have no conflict of interest.

## Ethical approval

All authors have read the manuscript and have given their consent for publication. This study does not involve any research with human participants or animals conducted by the authors.

## Data availability

All data supporting the findings of this study are included within the manuscript.

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