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Exploring the population structure and genetic diversity in apple germplasm using iPBS-retrotransposon markers

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Abstract: Apple (*Malus domestica* [Suckow] Borkh) holds global economic and cultural value, particularly in temperate regions. This study investigates the genetic variations among 52 apple accessions from three research centre's in Kazakhstan, employing the iPBS-retrotransposons marker system. Of the 35 markers initially screened, 12 highly polymorphic markers were selected for PCR amplification, producing 280 bands, 279 of which were polymorphic, yielding a polymorphism rate of 99.64%. Genetic diversity indices revealed notable variability with the effective number of alleles (ne = 1.655), Shannon's information index (I = 0.373), gene diversity (h = 0.549), and an average genetic distance of 1.2. Analysis of molecular variance (AMOVA) demonstrated that 97% of the genetic variation occurred within the population. STRUCTURE analysis divided the germplasm into two distinct populations and one unclassified population based on collection centers. Both the neighbor-joining tree and principal coordinate analysis (PCoA) supported these results, confirming the genetic separation into two groups. This study highlighted the significant genetic diversity among apple accessions, demonstrating the effectiveness of the iPBS-retrotransposons marker system. Additionally, the highest genetic distance (1.2) observed between the Tyulpan and Red Chief samples, positions these accessions as suitable and promising candidates for future breeding initiatives.

Key words: DNA markers, apple germplasm, genetic diversity, population structure, analysis of molecular variance

1. Introduction

Climate change poses a substantial threat to plants by altering key environmental variables, such as temperature, precipitation patterns, and frequency of extreme weather events (Al et al. 2023; Ali et al., 2024). These changes are having far-reaching effects on fruit production systems. Higher temperatures, disrupting the phenological cycle of fruit trees, which depend on finely tuned seasonal cues to synchronize processes such as bud break, flowering, and fruit sets. When these processes are misaligned, there can be significant reductions in both fruit yield and quality, as the trees fail to flower or fruit at optimal times. In addition, shifts in rainfall patterns and the increasing prevalence of drought conditions place further stress on fruit crops. Inadequate or erratic water availability hinders growth, weakens plant vigor, and reduces productivity. The combination of these factors with elevated atmospheric CO2 levels creates a complex environment that can exacerbate pest and disease pressures, further compromising fruit crop health and sustainability. Apple trees are particularly sensitive to these climate-related disruptions due to their specific physiological requirements. For example, apple trees rely on a period of winter chilling to break dormancy and initiate healthy growth in the spring. Insufficient chilling hours caused by warmer winters can result in delayed bud break, poor fruit set, and a significant decline in overall yield and quality. These climate-induced challenges are already impacting apple production in various regions, leading to fluctuations in supply and affecting the market

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both locally and globally (Delgado et al., 2021; González et al., 2023). In light of these challenges, it is imperative to develop strategies to mitigate the effects of climate change on fruit crops, including adaptive management practices and the breeding of climate-resilient varieties.

Global apple production, which amounts to approximately 93 million t annually (FAOSTAT, 2023), is a crucial element of the global food system. Apples' popularity as a snack fruit can be attributed to their extensive genetic diversity, successful cultivation in both the northern and southern hemispheres, diverse appearances, appealing aroma and taste, affordability, excellent transportability, reduced susceptibility to spoilage, and capacity for year-round storage (Akšić et al., 2021). Consequently, assessing the apple's popularity to climate change and exploring potential opportunities for cultivation under shifting climatic conditions is imperative. The extensive breeding of numerous cultivars has rendered the apple a highly adaptable species. In the northern hemisphere, apple cultivation predominantly occurs within the latitude range of 25° N to 52° N. Nevertheless, apple growing extends beyond this range in regions with favorable climatic conditions or those influenced by warm water currents (Vuković et al., 2023).

Globally, only 1% of agricultural land is dedicated to perennial fruit crops (Carranca et al., 2018). Specifically, a mere 53,000 square km are allocated to apple orchards. The leading apple producers are China (23 million t), the USA (4.5 million t), Poland (3 million t), Türkiye (2.3 million t), Italy (2.1 million t), France (2.1 million t), and Germany (2 million t) annually (Zhandybayev et al., 2023). These countries share a common trait: the intensification of apple production.

In Kazakhstan, apple cultivation is predominant among industrial plantations yet, 57% of the domestic apple market relies on imports (Maulenova et al., 2022). As of November 1, 2020, Kazakhstan had 147.6 thousand ha of perennial plantations. The majority of these fruit crops are located in three southern provinces: Turkestan Province (48%), Almaty Province (35%), and Zhambyl Province (7%). Most fruit plantations are operated by peasant and private farms, with apple trees occupying the largest area within the structure of fruit plantations in the Republic (Zhandybayev et al., 2023). Kazakhstan's favourable soil and climatic conditions make it a key region for fruit cultivation. The southern and southeastern parts of the Republic, where intensive orchards are prevalent, are divided into 4–5 natural-ecological zones, each characterized by distinct climatic conditions and topography (Ahmadi et al., 2019). The Tien Shan and Dzhungar Alatau mountain ranges in Kazakhstan are recognized as the primary centers for the origin and domestication of apple trees, as first identified by Vavilov in 1926. Genetic research corroborates that the local wild species, *Malus sieversii* M. Roem, is the principal progenitor of the cultivated apple tree (Velasco et al., 2010; Hernández-Carranza et al., 2016). The germplasm from these regions is highly valued for its rich genetic diversity, offering resilience against environmental stressors, pathogens, and pests, while also enhancing fruit quality (Dan et al., 2015; Omasheva et al., 2018). This diverse genetic base is essential for breeding programs aimed at improving apple varieties.

Extensive pomological surveys have identified a broad range of apple cultivated in Kazakhstan, including Adilet, Antonovka, Aport, Grushovka Vernenskaya, Kolzhatskoye, Maksat, Raika, Rashida, Renet Burkhardta, Sinap Almatinsky, Talgarskoe Zailiyskoye, and Zarya Alatau. Currently, sixty-six domestic and foreign varieties are officially listed in the State Register of the Republic of Kazakhstan (Omasheva et al., 2018). However, conventional breeding, based solely on phenotypic traits, presents limitations, as it is time-consuming and influenced heavily by environmental factors. These constraints highlight the need for integrating molecular techniques to accelerate the breeding processes (Altaf et al., 2024a; Poonam et al., 2023).

Molecular marker offers precision and efficiency by directly targeting genetic variations at the DNA level (Altaf et al., 2024b; Guizado et al., 2020; Yildiz et al., 2022). This approach not only conserves valuable genetic resources but also ensures the sustainable advancement of Kazakhstan's horticultural sector, aligning with modern agricultural practices and market demands (Nadeem et al., 2018; Yalinkiliç et al., 2024). The development of marker systems in plant genomics is significantly enhanced by the widespread presence of retrotransposons, which make them particularly effective in distinguishing apple clones, especially those arising from bud mutations. Traditional markers such as RAPD, AFLP, and SSR have demonstrated limited efficacy in differentiation (Venturi et al., 2006; Dhyani et al., 2015). Genetic similarity scores among apple genotypes typically range from 0.38 to 0.72, with Iranian apple genotypes displayed notable high genetic diversity and intraspecific variability, as revealed through cluster analysis. Goulão et al (2001) employed ISSR markers to analyze 41 varieties of *M. domestica*, uncovering an impressive polymorphism rate of 89.1%, which exceeded results from previous studies utilizing AFLP, RAPD, and SSR markers. SSR markers offer significant advantages due to their codominant inheritance, high allelic variation, and extensive genomic distribution (Liang et al., 2015). Recent research, including studies on Romanian apple trees (Giancarla et al., 2021) and *Malus sieversii* clones from Kazakhstan (Shadmanova et al., 2019), have confirmed the high genetic diversity and active genetic exchange within apple populations utilizing ISSR markers.

Retrotransposons are pervasive elements within plant genomes, capable of altering their location and copy number (Finnegan 1989). Among these, long terminal repeat (LTR) retrotransposons are more prevalent than non-LTR types (Kalender 2010). Kalendar et al. (2010) pioneered the inter-primer binding site (iPBS) marker system, which leverages both LTR and non-LTR retrotransposons. Due to their repetitive structures and regulatory signals, retrotransposons, play a significant role in the regulatory and structural dynamics of chromosomes (Huang et al., 2012). Their presence in all plant genomes, along with their ability to insert without eliminating original elements through replicative transposition underscores their importance (Arvas et al., 2023). DNA molecular markers, including LTR retrotransposons, are pivotal for understanding genetic diversity (Nadeem et al., 2018). This study aimed to explore the genetic diversity and population structure of 52 apple accessions from Kazakhstan using iPBS-retrotransposon markers. This investigation will provide a foundation for future apple breeding and genetic research.

2. Materials and methods

2.1. Germplasm details and DNA isolation

A total of 52 apple accessions were collected from the four different regions of Kazakhstan as plant material in this study. These samples were obtained from the Experimental Farm "Merkensky" LLP (Zhambyl region); "Zharkent Fruit" LLP (Zhetysu region); "Koktal" Peasant Farm, "Akniyet Agro Gardens" LLP, Regional Branch "Saryagash" of LLC "Kazakh Fruit and Vegetable Research Institute" and "Dala Fruit" LLP (Turkestan region); "Sady Zhetysu Trade" LLP, and Regional Branch "Talgar" (Pomological Garden) of LLC "Kazakh Fruit and Vegetable Research Institute" (Almaty region) (Table 1). Fresh, young, and healthy leaf tissues were taken for DNA extraction, which was performed using the CTAB protocol proposed by Doyle and Doyle (1990), with minor modifications (Baloch et al. 2023). The concentration of DNA was evaluated with the help of a NanoDrop (TM 2000/2000c ND-2000, Thermo Fisher Scientific Inc., Wilmington, DE, USA) and further confirmed by agarose gel electrophoresis (i.e. 0.8% agarose gel). The final DNA concentration was adjusted to 5 ng/µL for Polymerase chain reactions (PCR) and stored at – 20 °C.

Table 1. Passport data of studied apple germplasm utilized in this investigation.

S. No	Accessions	Country of origin	Apple accession collection centers	Geographic coordinators
1	Red Delicious	USA	Zhambyl	N 42°48′.584″ E 73°10′.387″
2	Golden Delicious	USA		
3	Star Crimson	USA		
4	Babushkino	Russia (old cultivar)		
5	Granny Smith	Australia	Zhetysu	N 44°03′10.8″ E 79°32′14.1″
6	Fuji	Japan		
7	Pink Lady	Australia		
8	Gala	New Zealand		
9	Scarlet Spur	USA		
10	Jeromine	USA		
11	Golden Delicious	USA		
12	Gala	New Zealand	Turkestan region	N ‘42,55699° E 69,52156°
13	Golden Delicious	USA	Turkestan	N 41°29′2.69″ E 70°31′11.46″
14	Red Chief	USA		
15	Idared	USA	Turkestan	N 41°36′8.594″ E 69°21′58.013″
16	Vesna	Russia	Turkestan Institute"	N 41°32′2.545″ E 69°21′36.069″
17	Idared	USA	Turkestan	N 41°29′2.69″ E 70°31′11.46″
18	Konfetnoe	Russia	Turkestan	N 41°36′8.594″ E 69°21′58.013″

Table 1. (Continued.)

19	Maminy Stakanchiki	Russia	Turkestan	N 41°29′2.69″ E 70°31′11.46″
20	Golden Delicious	USA	Turkestan	N 41°32′2.545″ E 69°21′36.069″
21	Star Crimson	USA	Turkestan	N 41°32′2.545″ E 69°21′36.069″
22	Reinette Simirenko	Ukraine	Turkestan	N 41°32′2.545″ E 69°21′36.069″
23	Golden Delicious	USA	Turkestan	N ‘42,55699° E 69,52156°
24	Golden Delicious	USA	Turkestan	N 41°36′8.594″ E 69°21′58.013″
25	Star Crimson	USA	Turkestan	N 41°36′8.594″ E 69°21′58.013″
26	Papirovka	Russia	Turkestan	N 41°32′2.545″ E 69°21′36.069″
27	Golden Delicious	USA	Turkestan	N 41°29′2.69″ E 70°31′11.46″
28	Reinette Simirenko	Ukraine	Turkestan	N 41°29′2.69″ E 70°31′11.46″
29	Aport	Kazakhstan	Turkestan	N 41°29′2.69″ E 70°31′11.46″
30	Pink Lady	Australia	Turkestan	N 41°36′8.594″ E 69°21′58.013″
31	Star Crimson	USA	Turkestan	N 41°29′2.69″ E 70°31′11.46″
32	Gala	New Zealand	Turkestan	N 41°36′8.594″ E 69°21′58.013″
33	Divo	Russia	Almaty	N 43°31′48.31″ E 78°11′48.09″
34	Sarkhyt	Kazakhstan	Almaty	N 43°17′27″ E 77°12′15″
35	Fuji	Japan	Almaty	N 43°31′48.31″ E 78°11′48.09″
36	Saltanat	Kazakhstan	Almaty	N 43°17¢27¢¢ E 77°12¢15″
37	Korey	Japan	Almaty	N 43°31′48.31″ E 78°11′48.09″
38	Gala	New Zealand	Almaty	N 43°31′48.31″ E 78°11′48.09″
39	Danalyk	Kazakhstan	Almaty	N 43°17′27″ E 77°12′15″
40	Talgarskoye	Kazakhstan	Almaty	N 43°17′27″ E 77°12′15″
41	Tyulpan	Kazakhstan	Almaty	N 43°17′27″ E 77°12′15″
42	Pestrushka	Russia	Almaty	N 43°17′27″ E 77°12′15″
43	Golden Delicious	USA	Almaty	N 43°31′48.31″ E 78°11′48.09″
44	Zarya Alatau	Kazakhstan	Almaty	N 43°17′27″ E 77°12′15″

Table 1. (Continued.)

45	Williams' Pride	USA	Almaty	N 43°17′27″ E 77°12′15″
46	Ainur	Kazakhstan	Almaty	N 43°17′27″ E 77°12′15″
47	Sinap Alma-Atinsky	Kazakhstan	Almaty	43°17′27″N 77°12′15″E
48	Quinte	Canada	Almaty	N 43°31′48.31″ E 78°11′48.09″
49	Star Eliest	USA	Almaty	N 43°31′48.31″ E 78°11′48.09″
50	Landsberger Reinette	Germany		
51	Aport	Kazakhstan	Almaty	N 43°17′27″ E 77°12′15″
52	Red Delicious	USA	Almaty	N 43°31′48.31″ E 78°11′48.09″

2.2. iPBS-retrotransposon-based PCR amplifications

The iPBS-retrotransposons markers were utilized in this work to evaluate the genetic diversity of the apple germplasm. All the primers utilized in this study were sourced from a study conducted by Kalendar et al. (2010). Four apple samples were selected randomly and screened using 33 primers. The 12 primers that showed the highest level of polymorphism among the 33 screened primers were chosen for PCR amplification of all 52 apple accessions (Table 2). A PCR reaction mixture with a volume of 20 µL was made by adding 5 ng of template DNA and a PCR mix. The PCR mix contained 1X PCR buffer (Thermo Scientific), 0.6 mM concentration of 18-nt primers or 1 mM concentration of 12–13-nt primers, 0.2 mM concentration of each type of deoxyribonucleotide triphosphate (Thermo Scientific, Waltham, MA, USA), 2 mM concentration of MgCl2, and 0.2 U of Taq DNA polymerase (Thermo Scientific). The PCR process involved an initial predenaturation step at a temperature of 95 °C for 3 min. This was followed by 30 cycles, each consisting of a denaturation step at 95 °C for 15 s, an annealing step at a temperature ranging from 50 to 65 °C (depending on the primer) for 1 min, and a final extension step at 72 °C for 5 min. A 2% agarose gel electrophoresis, with a weight/volume ratio of 0.5 x Tris-borate-EDTA (TBE) buffer, was conducted to separate PCR amplicons for 155 min. Next, ethidium bromide was meticulously employed to dye the gel for enhanced viewing using the Imager Gel Doc XR + system (Bio-Rad, Hercules, CA, USA). A ladder with a size of at least 100 base pairs was utilized as a reference for determining the molecular weight.

2.3. Statistical analysis

PCR product scoring was performed using a binary system, where a score of 0 indicated the absence of a band, while a score of 1 indicated their presence. Diversity attributes such as genetic diversity (He), Shannon's information index (I), and the effective number of alleles (Ne) were calculated using PopGen software version 1.32 (Yeh et al., 1997). The polymorphism information content (PIC) was determined using the formula PIC = 2fi(1-fi), as described by Roldán-Ruiz et al. (2000), where fi represents the frequency of present alleles for a given marker, and '1-fi' denotes the frequency of absent alleles. To further elucidate the genetic relationships and population structure within the studied germplasm, multivariate analyses were performed. Principal coordinates analysis (PCoA) and analysis of molecular variance (AMOVA) were conducted using the GeneALEx version 6.5 software package (Peakall and Smouse, 2006). The unweighted pair-group arithmetic mean (UPGMA) was constructed using R statistical software (version 3.4.1).

The genetic structure of the studied germplasm was assessed using a Bayesian clustering model in STRUCTURE. The Bayesian clustering analysis commenced with an initial burn-in period of 50,000 iterations, followed by 300,000 Markov Chain Monte Carlo (MCMC) iterations. No prior information regarding the origin or population affiliation of individuals was provided. To estimate the population structure, parameters were set for 10 independent runs for each potential population cluster. The appropriate number of genetic clusters (K) in the analysis was determined based on the criteria proposed by Evanno et al. (2005).

3. Results

The apple germplasm was characterized using 12 polymorphic iPBS-retrotransposon primers. These primers produced a total of 280 strong and distinct bands, averaging 23.33 bands per primer across 52 apple accessions. Polymorphism was detected in 279 (99.64%) of the 280 bands, with an average of 23.25 bands per primer (Table 3). The mean polymorphism rate was 99.64%, ranging from 95.65% for primer 2389 to 100% for the other primers used. Primer 2380 produced the lowest bands (10). Additionally, 11 primers (2074, 2245, 2402, 2383, 2228, 2381, 2401, 2380, 2075, 2230, and 2251) exhibited 100% polymorphism, while primer 2389

Table 2. Descriptive details of iPBS-retrotransposon markers used in elucidating genetic diversity among Apple germplasm.

Primer name	Sequence	Annealing temperature (°C)
2074	GCTCTGATACCA	50
2389	ACATCCTTCCCA	50
2380	CAACCTGATCCA	50
2381	GTCCATCTTCCA	50
2075	CTCATGATGCCA	50
2383	GCATGGCCTCCA	53
2245	TAGGCTCGGATGCCA	50
2230	AGGCGTCTGATACCA	53
2402	TCTAAGCTCTTGATACCA	50
2228	CATTGGCTCTTGATACCA	53
2401	AGTTAAGCTTTGATACCA	53
2251	GAACAGGCGATGATACCA	53

Table 3. Genetic diversity parameters estimated using iPBS-retrotransposon markers in Apple germplasm

Primer	Total bands	Polymorphic bands	% Polymorphism	Ne	h	I
2074	29	29	100	1.68	0.39	0.57
2389	23	22	95.65	1.73	0.41	0.6
2245	21	21	100	1.66	0.38	0.57
2402	20	20	100	1.64	0.37	0.55
2383	20	20	100	1.63	0.37	0.55
2228	25	25	100	1.69	0.39	0.56
2381	22	22	100	1.58	0.33	0.49
2401	26	26	100	1.58	0.34	0.51
2380	10	10	100	1.63	0.35	0.52
2075	24	24	100	1.67	0.38	0.55
2230	29	29	100	1.71	0.4	0.58
2251	31	31	100	1.66	0.37	0.54
Mean	23.33	23.25	99,64	1.655	0.373	0.549
Total	280	279				

produced 22 out of 23 bands with a polymorphism rate of 95.65%. The maximum number of effective alleles (Ne) was 1.73 for primer 2389, followed by 1.71 for primer 2230, 1.69 for primer 2228, 1.68 for primer 2074, and the minimum Ne of 1.58 for primers 2381 and 2401. The average Ne across all iPBS-retrotransposon primers was 1.655. The highest Shannon's information index (I) was 0.41 for primer 2389, while the lowest was 0.33 for primer 2381, with an average I of 0.373 across all primers. The highest gene diversity (h) was 0.60 for primer 2389 and the lowest was 0.49 for primer 2381, with a mean gene diversity of 0.549 for all tested primers. The Nei's higher genetic distance (1.2) was observed between the Tyulpan and Red Chief samples, while the smallest genetic distance (0.089) was found between the Star Crimson and Red Delicious accessions from the Zhambyl region, as well as between the Danalyk and Talgarkoye accessions from the Almaty region.

The model-based clustering algorithm implemented in the structure analysis partitioned the studied apple germplasm into two distinct populations. The first population comprised 17 accessions, constituting 32.69% of the total accessions, while the second population consisted of 31 accessions, representing 59.61% of the total accessions. Additionally, four accessions (7.69% of the total) remained unclassified and did not belong to either of the identified populations. This population structure was primarily influenced by the geographical regions from which the accessions were collected (Figure 1). Corroborating these findings, the neighbor-joining tree analysis also divided the entire germplasm into two distinct groups (Figure 2). Furthermore, the principal coordinates analysis (PCoA) separated the accessions into distinct clusters based on their geographic localities (Figure 3), supporting the results obtained from the UPGMA and structure-based clustering approaches. The analysis of molecular variance (AMOVA) revealed that 97% of the observed genetic variation was attributable to differences within populations in the studied germplasm (Table 4).

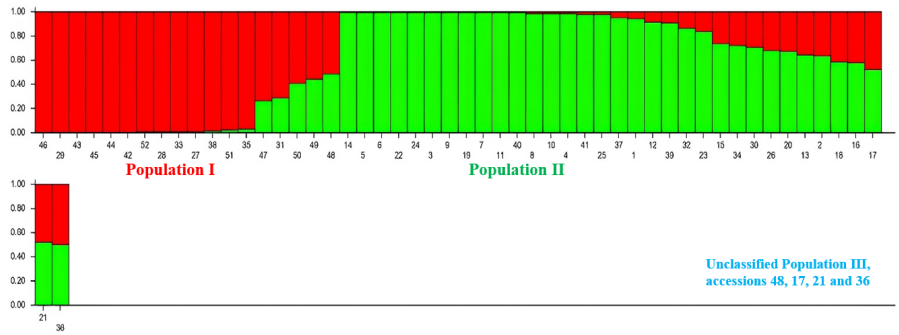

Figure 1. Population structure analysis of 52 apple accessions through iPBS-retrotransposon marker system.

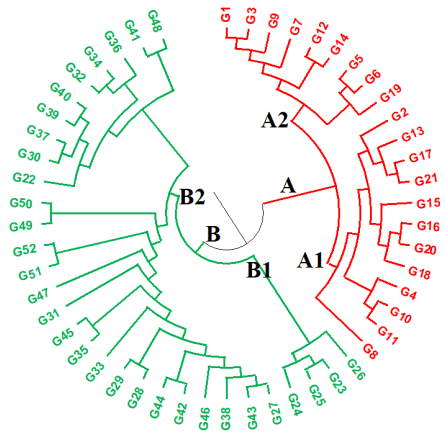

Figure 2. Neighbour-Joining Analysis of the evaluated 52 apple accessions through the iPBS-retrotransposon marker system.

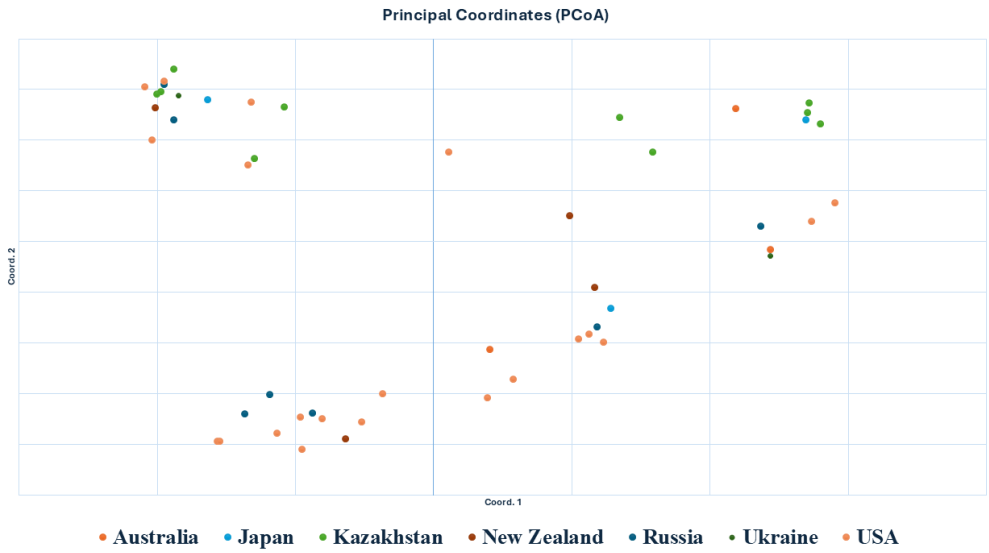

Figure 3. PCoA Analysis of the evaluated studied apple germplasm through the iPBS-retrotransposon marker system.

Table 4. The analysis of molecular variance (AMOVA) for the studied apple germplasm with iPBS-retrotransposons markers.

Source	df	SS	MS	Est. Var.	%
Among populations	6	484.270	80.712	2.216	3%
Within populations	43	2876.050	66.885	66.885	97%
Total	49	3360.320		69.101	100%

4. Discussions

Understanding the genetic diversity within a plant's germplasm is crucial for uncovering the species' inherited traits and optimizing breeding efforts (Iqbal et al., 2023). The worldwide trade of apple plants has increased because of the rising world population in recent years. Understanding the molecular characterization of apple plants is crucial for breeders to make well-informed choices when selecting parents for breeding initiatives (Laurens et al., 2018). Various DNA-based molecular markers have been used for the assessment of genetic variation in apple germplasm (Kuras et al. 2013; Gencer and Serce 2023). The iPBS-retrotransposons marker system is universally applicable and does not require sequence knowledge, making it a valuable tool for genetic studies. However, when using the LTR retrotransposons as genetic markers, prior knowledge of their sequence is necessary to ensure accurate identification and analysis (Demirel et al., 2018). These prerequisites enable precise targeting and enhance the efficacy of retrotransposons-based markers in plant studies (Baloch et al., 2022; Baran et al., 2023), including wheat (Demirel et al., 2024), Chestnut (Orhan and Kara, 2023), and grapes (Sümbül et al., 2024), respectively.

Recently, Gencer and Serce (2022), conducted a study to evaluate the genetic variation among the 60-apple accession in Türkiye and found higher polymorphism (94%) and higher genetic diversity among the studied germplasm. However, the polymorphism observed in our study was higher than the Gencer and Serce (2020). Dar et al. (2019) employed 29 SSR markers to asses genetic diversity among the 19 apple accessions in the Kashmir. During the study, various parameters such as PIC, resolving power, and marker index (MI) were computed. A total of 218 polymorphic bands were obtained ranging from 03 to 14 alleles per primer, with an average of 7.51 alleles per SSR marker. To examine the genetic variation among the 29 apple accessions, Raja et al. (2022) employed a total of 85 SSR markers. Najar et al. (2023) reported 77 number of alleles with an average polymorphism percentage (APP) of 87.5%, a PIC of 0.71, and a resolving power (RP) of 3.58. The genetic diversity indices, including the average number of alleles (Na = 1.67), Shannon's information index (I = 0.43), the effective number of alleles (Ne = 1.47), and expected heterozygosity (He = 0.3), indicated a moderate level of genetic diversity across the germplasm. Dar et al. (2020) utilized a set of 10 RAPD markers to assess the diversity across 19 apple cultivars. The selective ability of each random primer was assessed based on variables such as PIC, RP, and marker index. There were 70 polymorphic bands were detected, with a polymorphism ratio of 83.33%. Our results were higher than the earlier above-mentioned studies that showed the applicability, effectiveness, and robustness of the iPBS-retrotransposons marker system.

Clustering methods such as STRUCTURE, principal coordinate analysis (PCoA), and neighbor-joining analysis have been employed to understand the genetic diversity and population structure of the studied germplasm. Previous studies have found that the model-based structure application was more reliable and provided richer insights compared to other approaches (Alsaleh et al., 2022). In this study, the iPBS-retrotransposon marker system was used to look at the population structure of apple germplasm. The results showed that the accessions collected from different regions were divided into two populations: population I, population II, and one an unclassified group Population I comprises 17 apple accessions (32.69% of the total population) with notable representation from Kazakhstan, the USA, Russia, and New Zealand. This clustering strongly supports the hypothesis that Malus sieversii, the wild ancestor of the domesticated apple, originating in the Tian Shan mountains of Kazakhstan, played a foundational role in apple domestication (Brite, 2021). The presence of accessions such as Aport and Ainur (Kazakhstan) in Population I underscores the region's importance as the genetic source of key domesticated traits. The inclusion of modern cultivars from the USA (e.g., Golden Delicious, Red Delicious) in the same cluster reflects their derivation from germplasm with ancestral links to Central Asia. These cultivars underwent genetic improvement during their dissemination to Western breeding programs, integrating traits that trace back to the primary domestication site.

Population II, containing 31 accessions (59.61% of the total population), represents a wider geographic distribution with contributions from the USA, Russia, Australia, New Zealand, Japan, and Ukraine. This population showcases the influence of hybridization and adaptation during the apple's global spread. The high representation of accessions from the USA (e.g., Red Delicious, Star Crimson, Pink Lady) reflects extensive breeding efforts in North America, which leveraged diverse

genetic material, including hybrids with Malus sylvestris (European crabapple) and other regional apple species. The presence of accessions from Japan (Fuji, Korey), Russia (Babushkino, Vesna), and Kazakhstan (Danalyk, Talgarskoye) in this population highlights the apple's adaptability to varying climates and cultural preferences. The clustering suggests that these accessions retain genetic signatures from the original domestication events but have undergone localized selection for traits like cold tolerance, fruit quality, and disease resistance. Population structure divided the whole germplasm according to their collection centres and proved their applicability and effectiveness, therefore, we used here it as a benchmark.

The PCoA and NJ analysis divided the apple germplasm into two primary groups and supported the results of structure. In NJ, Group I (59.61%, 31 accessions) and Group II (40.38%, 21 accessions). Group I was further divided into subgroups I-A and I-B, with I-A containing diverse accessions like Reinette Simirenko (Ukraine), Aport (Kazakhstan), Fuji (Japan), and Golden Delicious (USA), reflecting genetic contributions from the Central Asian domestication center and global breeding programs. Subgroup I-B included accessions like Pink Lady (Australia) and Sarkhyt (Kazakhstan), indicating regional adaptations.

Group II, similarly split into II-A and II-B, included accessions such as Red Delicious (USA), Granny Smith (Australia), and Maminy Stakanchiki (Russia) in II-A, while II-B featured Golden Delicious (USA) and Babushkino (Russia). These clusters highlight the role of Central Asia in apple domestication and subsequent diversification through hybridization and regional breeding efforts. The analysis emphasizes the genetic diversity within apple germplasm, shaped by historical and modern breeding. Our research highlights the importance of iPBS-retrotransposon markers in revealing the genetic diversity and population structure of apple germplasm, a crucial part of breeding initiatives. The notable polymorphism rates and distinct population stratification offer significant insights into the genetic composition of apple accessions from Kazakhstan. These results are essential for future breeding initiatives, particularly in light of climate change, which necessitates the creation of hardy and productive apple varieties. The classification of accessions into separate populations according to geographic origin highlights the impact of local environmental factors on genetic diversity. This genetic diversity is a crucial asset for apple enhancement efforts, guaranteeing the sustainability and adaptation of apple agriculture across diverse locations. Subsequent research, incorporating supplementary molecular markers and a broader array of accessions, is advised to augment the comprehension of genetic linkages and improve the accuracy of apple breeding initiatives.

Conclusion

In conclusion, this study revealed substantial genetic diversity in 52 apple accessions using iPBS retrotransposons markers. The high polymorphism rate (99.64%) and considerable genetic variation among accessions highlight the effectiveness of the iPBS markers system for molecular characterization. The population structure analysis grouped the germplasm into two distinct populations, providing information about genetic linkages and regional distribution. These finding enhances our knowledge of apple germplasm diversity and highlight the importance of molecular markers for breeding programs focused on improving apple varieties to meet the demands of modern agricultural challenges.

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Author contributions

Methodology: AM, AA, and MAN; software: MAN; validation: MAN and FSB; formal analysis: MAN and MTA; investigation: FSB; data curation: AK and BK; writing—original draft preparation: AA, MTA, and DK; review and editing: FSB, and MAN; supervision: FSB and MAN. All authors have read and agreed to the published version of the manuscript.

Data availability

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

Declarations

Conflict of interest: The authors declare that they have no conflict of
interests.

Ethical approval: This manuscript does not contain any experiments involving human or animal participants.

Consent to participate: Not applicable.

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