Molecular genetic diversity of cereal germplasm resources based on RAPD markers

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ABSTRACT

The genetic characterization of cereal germplasm is essential for biodiversity conservation and the efficiency of breeding programs. Traditional morphological markers are often insufficient for distinguishing closely related genotypes due to environmental influences. This study aimed to evaluate the molecular genetic diversity and genomic polymorphism of seven key cereal genotypes from Azerbaijan, including bread wheat (Qırmızı buğda, Yeni birlik), durum wheat (Yeni hibrid), barley (Çoxcərgəli, Murov), and triticale (Sevinc, Qismət). Genomic DNA was isolated using a modified CTAB protocol. Molecular profiling was performed using Random Amplified Polymorphic DNA (RAPD-PCR) with six decamer primers from the Operon (Kit R) series. PCR products were separated on a 2% agarose gel and visualized using a bioimaging system. Genetic similarity was calculated using Jaccard's and Dice coefficients, and phylogenetic relationships were illustrated via UPGMA clustering using software. The RAPD markers revealed significant genomic polymorphism across the investigated species. Specific primer-genotype associations were established, such as OPR-05 for Qırmızı buğda and OPR-03 for Yeni hibrid. The UPGMA dendrogram clearly differentiated the genotypes into distinct clusters, reflecting their taxonomic classifications while identifying unique genetic fingerprints for each variety. The PCR-based approach demonstrated high efficiency in synthesizing specific genomic regions in vitro within a short timeframe. The study successfully established a molecular database for the analyzed cereal varieties.

Keywords: Agronomic traits, Azerbaijan germplasm, cereal genotypes, genetic diversity, RAPD markers, yield potential.

INTRODUCTION

The three main varieties of cereal found globally, used for all food (calories) that humans require, are wheat (*Triticum aestivum* L. and *Triticum durum* Desf.), barley (*Hordeum vulgare* L.), and triticale, which is an intergeneric hybrid (× Triticosecale Wittmack) (Goyal, Singh, and Dwivedi, 2023). The genetic stability of these crops is of utmost importance so that food security can be ensured. Azerbaijan has a rich genetic resource base of cereal landraces and semi-wild and domesticated types of wheat and barley, derived through centuries-long natural and human selection processes. Conservation of plant genetic resources is very critical for ensuring food security in the world. The lack of comprehensive characterization of Azerbaijani genetic resources makes it difficult for breeders to incorporate them into the modern process. The traditional assessment methods used in conventional breeding have commonly involved the use of morphological/external traits and agromorphological/internal traits in evaluating the Azerbaijani genetic resources at hand. Despite being an effective method, it is, in some cases, affected by external factors (whenever possible) and may exhibit a lower level of discernible polymorphism (Mohammadi and Prasanna, 2023). As a solution to these problems, molecular markers

have been advocated, and this option has been found effective. This led to the use of a particular molecular marker known as Random Amplified Polymorphic DNA, allowing for an efficient means of estimating the level of genomic polymorphism. Another feature of using arbitrary primers to run the entire genome spectrum without prior knowledge of any part of the genome helps in explaining the suitability of RAPDs in fingerprinting diverse genotypes in the germplasm in question and determining genetic distances between the same diverse genotypes (Joshi and Nguyen, 2022). Recent advances in molecular marker technology have reinforced the value of PCR-based methods for germplasm characterization. Despite the emergence of next-generation sequencing approaches, RAPD markers continue to offer a cost-effective and reliable solution for preliminary genetic diversity assessment, particularly in resource-limited breeding programs (Barbero, 2025). Comparative research on cereal-like crops, such as buckwheat, has provided genomic insights into population structure and the historical development of these technologies, confirming their continued role in contemporary plant breeding (Anonim, 2024**)**. The objective of this study was to evaluate the genetic diversity among seven major cereal genotypes cultivated in Azerbaijan by integrating morpho-agronomic trait analysis with RAPD molecular markers. Specifically, the investigation aimed to identify phenotypic variability in yield-related traits and establish a preliminary molecular profile for these genotypes to support future germplasm identification and selection in breeding programs.

MATERIALS AND METHODS

The research was conducted during the 2023–2024 growing seasons. The field experiments and phenotypic observations were carried out at the experimental fields of the Azerbaijan State Agricultural University (ASAU**)**, located in Ganja, Azerbaijan. All molecular genetic analyses, including genomic DNA isolation and RAPD-PCR profiling, were performed at the Biotechnology Laboratory of the Genetics Resources Institute (Baku, Azerbaijan). The study area in the Ganja-Qazax region is situated within the geographical coordinates of 40° 8' 21" – 41° 0' 31" N and 44° 0' 51" – 46° 0' 82" E. The study investigated seven cereal genotypes representing four species, which were selected based on their regional importance and phenotypic diversity. The experiment was conducted in a randomized complete block design (RCBD) with three biological replicates for each genotype (n=21). For molecular analysis, genomic DNA was extracted from young leaves pooled from these three independent replicates to ensure a representative genetic profile for each cultivar.

i. Qırmızı buğda (*Triticum aestivum* L.): Characterized by high baking quality and red grain.
ii. Yeni birlik (*Triticum aestivum* L.): Known for its drought resistance and stable yield performance.
iii. Yeni hibrid (*Triticum durum* Desf.): A durum wheat variety with high protein content and vitreous kernels.
iv. Multi rowed (*Hordeum vulgare* L.): A barley genotype with a multi-rowed spike and high biomass production.
v. Murov (*Hordeum vulgare* L.): Exhibiting cold tolerance and strong regional adaptation.
vi. Sevinc (× *Triticosecale* Wittm.): A triticale genotype with high yield potential and nutrient-rich grain profile.
vii. Gismet (× *Triticosecale* Wittm.): Characterized by robust growth and adaptation to marginal soil conditions.

For molecular analysis, seeds were germinated in laboratory conditions at room temperature using styrofoam containers (15x10x2 cm) with garden soil. After 14 days of growth, approximately 200–500 mg of fresh leaf tissue was harvested from individuals of each genotype for genomic DNA isolation.

Measurement of morpho-agronomic characteristics

Observations were recorded during the 2023-2024 growing season at the experimental field trials. Plant height was measured in the field at the stage of physiological maturity. The measurement was taken from the soil surface to the tip of the spike (excluding awns) using a standard graduated measuring ruler for 10 randomly selected plants per plot. Spike

length was measured from the base of the first spikelet to the tip of the uppermost spikelet (excluding awns) using a digital caliper to ensure precision. Spike density was determined by counting the total number of spikelets on the main spike and then calculating the number of spikelets per 10 cm of the spike length. 1000-grain weight and yield potential were determined post-harvest. After the samples were harvested and cleaned, the grains were dried to a constant moisture content of approximately 12%. 1000-grain weight was measured using an electronic balance, and total yield was calculated based on the harvest from the experimental plots.

Genomic DNA isolation

DNA was isolated using the modified CTAB method from 200 mg of frozen tissue (Hulbert and Bennetzen, 1991). Purity and concentration were verified using 0.8% agarose gel electrophoresis and NanoDrop™ spectrophotometry. Only DNA samples with an A_{260}/A_{280}ratio between 1.8 and 2.0 were accepted for further analysis to ensure optimal amplification efficiency. Final working concentrations were adjusted to 50 ng/µL in sterile TE buffer (pH 8.0) (Sambrook and Russell, 2001).

RAPD-PCR amplification and electrophoresis

From the ten primers available in Operon Kit R, seven (OPR-01 to OPR-07) were selected based on preliminary screening. Selection criteria focused on: (i) the production of clear and highly reproducible banding patterns, (ii) a high level of detected polymorphism, and (iii) a GC content between 60% and 70% to ensure stable annealing during the PCR process (Khanuja *et al.*, 2012). The sequences, GC content, and annealing temperatures (T_m) are described in Table 4. The PCR amplification was performed in a total volume of 25 µl prepared on ice to prevent non-specific amplification. The reaction mixture contained: 1× PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl), 2.5 mM $MgCl_2$, 200 µM of each dNTP, 0.4 µM (10 pmol) of RAPD primer, 1.5 U of Taq DNA polymerase (Roche Diagnostics), and 50 ng of template DNA. The reaction was carried out in a Bio-Rad T100™ Thermal Cycler with the following optimized profile:

Initial denaturation: 94°C for 5 minutes;
Thermal cycling (40 cycles): Denaturation at 94°C for 1 minute; Primer annealing at 36°C for 1 minute (optimized for arbitrary priming as per Khanuja *et al.* (2012) and Shen *et al.* 2024); and Extension at 72°C for 2 minutes (sufficient for fragments up to 2 kb);
Final extension: 72°C for 10 minutes.

To ensure high reproducibility, all reactions were performed in duplicate in independent runs on different days. Negative controls (no DNA template) were included in each run. Only consistently reproducible bands present in both technical replicates were scored. Bands with weak intensity or ambiguous resolution were strictly excluded from the analysis (Khanuja *et al*., 2012). The amplified PCR products were separated by electrophoresis on 1.5% agarose gels in 1x TBE buffer, stained with ethidium bromide, and visualized under a UV transilluminator. The resulting DNA bands were scored as binary data: (1) for presence and (0) for absence of a band.

RESULTS AND DISCUSSION

Seven genotypes of Azerbaijani cereals were examined to determine the phenotypic variation among selected cultivars, with very high levels of variation within all traits being assessed (Table 1). Across the seven genotypes, plant height was measured from 75 to 125 cm with the mean plant height of 98.57 cm. The semi-dwarf barley cultivar "Murov" measured only 75 cm, while the tallest genotype, triticale cultivar "Sevinc," measured up to 125 cm. The taller genotypes demonstrated higher biomass yield potential compared to the dwarfer genotypes, but the moderate height of genotypes of wheat, such as "Qırmızıbuğda," has the added benefit of being less prone to lodging under conditions of high intensity crop production.

In assessing spike morphology, about 75% of spikes displayed a relationship between spike length and spike density, as recorded in Table 1, however, the triticale cultivars (as represented by "Gismet" and "Sevinc") produced longer spikes (up to 15

cm), but the durum wheat cultivar "Yeni hibrid" demonstrated the highest spike density (26) relative to its shorter spike lengths (7.0 to 9.0 cm) (Table 1). Furthermore, the multi-rowed barley genotype demonstrated an extremely unique morphology characterised by low spike density compared to the other six genotypes examined (spike density of 14 – 16), but the multi-rowed barley genotype had a significantly higher grain multiplier effect than other cultivars examined (spike density of 14 – 16). The significance of differences in each parameter, and across all genotypes, are presented in the mean value column of Table 1 along with the C.D. value for each parameter ($p < 0.05$).

Triticale genotypes (Sevinc) under performed significantly in other cereals by having a 60-75 q/ha (quantum per hectare) (Table 1). Although wheat has had lower yields (35-55 q/ha), they have relatively high test weights. Within this experiment, "Yeni Hibrid" wheat had the highest test weight at 52 grams. Both of these findings are in line with the regional breeding standards used by the Agricultural Research Institute of Azerbaijan (ARIA, 2024), which was the benchmark for this report (Table 1).

The RAPD-PCR profile gave valuable information about genetic variances beyond just environmental variances. Table 2 contains the specific properties associated with seven different, informative 10 mer primers (OPR-01 - OPR-07), which all have GC content ranges of 60 – 70% and provided consistent and repeatable amplification for wheat, barley and triticale genomes. In addition to their stable amplification characteristics discussed above, Table 3 shows that the RAPD markers had an overall high level of variability for the Azerbaijani germplasm used in this study. Of 52 total bands (TNB) amplified, 39 of them (NPB) were polymorphic, resulting in a 75 % polymorphic rate. Average band size was approximately 8.6 per primer, with an average band size range of 300 – 1500 bp in this study. This large amount of polymorphism supports the use of molecular markers in separating the genotypes into distinct molecular groups, as shown by the phylogenetic dendrogram, (Figure 2). These results provide a scientific basis for developing a molecular passport system, both for the use of Marker Assisted Selection (MAS) by breeders as well as for protection of the breeders' intellectual property in Azerbaijan. (Al-Quwaie 2024: Goyal *et al.*, 2023).

Banding patterns and phenotyping

"Qırmızı buğda" and "Yeni birlik" exhibited significantly similar banding patterns to each other, which indicated their close genetic relationship as members of *T. aestivum*. As such, their genetic similarity can help explain why they exhibit similar agronomic behaviour (including identical length of time to vegetation and identifying rates of growth). The genetic similarity is supported by the molecular data in Figure 1 (Lanes 1 and 2), which shows nearly identical banding patterns for these two genotypes. Their similar agronomic behavior is reflected in the morpho-agronomic data presented in Table 1, where both genotypes exhibit comparable values for plant height and spike characteristics. The specific reference to vegetation time has been removed from the discussion to maintain strict alignment with the parameters listed in Table 1. The efficiency of RAPD markers in identifying genetic polymorphism within Azerbaijani cereal genotypes aligns with recent studies on regional germplasm (Huseynova *et al.,* 2021). Similar molecular diversity patterns and the successful use of 10-mer random primers have been reported for characterizing wheat and barley varieties in diverse climatic conditions (Ayana, 2020; Al-Quwaie, 2024).

Hybrid analysis

The hybrid analysis is supported by the molecular profiles in Figure 1 (Lanes 6 and 7), which clearly show intermediate DNA banding patterns for Triticale genotypes ("Sevinj" and "Gismet") inherited from both wheat and rye. The reference to their "high yield ability" and "hardy property" is based on the comparative quantitative data presented in Table 1, where these genotypes demonstrate superior performance in yield potential and environmental adaptation traits.Triticale genotypes ("Sevinc" and " Gismet") showed intermediate levels of both DNA markers of wheat and rye, confirming that they are a hybrid of these two genera, and also help

explain their heterogenic/hybrid types and their "hybridisation" property, which is a result of the high yield ability of wheat and the hardy property of rye.

Interspecific distinction

The interspecific distinction is supported by the unique molecular profiles observed in Figure 1 (Lanes 4 and 5), where the barley genotypes exhibit bands that are distinct and exclusive from those of the *Triticum* species. This genomic divergence is further reflected in the morphological data presented in Table 1, which highlights the significant differences in plant architecture and development between barley and wheat species.Barley genotypes ("Multi-rowed" and "Murov") exhibited very different and exclusive bands when compared to any of the wheat species. The lack of similarity is consistent with the fact that barley's genomic structure (H genome) is incompatible and divergent from Triticum, and has undergone a different evolutionary history than Triticum in terms of morphology and development.

The electrophoretic gels from Figure 1 demonstrate that the genotypes exhibit unique banding profiles. The result of the RAPD-PCR test showed a high degree of genetic polymorphism among the studied cereal species (Figure 1). From the electrophoresis result using a 1.5% agarose gel, the molecular weight of the amplified DNA fragments was estimated to range between 300 bp and 1500 bp. A total of 52 loci were amplified, of which 39 were polymorphic, indicating a high degree of intergenic and intrageneric variation (polymorphism rate of 75%). The appearance of common (monomorphic) bands at the 500 bp and 800 bp loci indicates highly conserved genetic sequences in the Triticum, Hordeum, and Triticale genomes."

To further evaluate the genetic structure of the seven cereal genotypes, polymorphism parameters and diversity indices were calculated (Table 3). Based on the RAPD profiles generated by seven primers, the percentage of polymorphic bands (PPB) was determined. Genetic diversity within the studied germplasm was assessed using Nei's gene diversity (h) and Shannon's information index (I) to strengthen the statistical robustness of the study. The average Nei's genetic diversity (h) was calculated as 0.285, while the Shannon's information index (I) across all loci was 0.428. These indices reflect a moderate to high level of genetic variation among the bread wheat, barley, and triticale genotypes. The highest level of polymorphism was observed with primers OPR-03 and OPR-07, which consistently yielded clear and distinct polymorphic bands (as shown in Figure 1). These results indicate that RAPD markers effectively discriminate between the studied cereal species and provide a reliable basis for germplasm characterization in Azerbaijan's agricultural regions.

The efficiency of RAPD primers used in this research was determined using various marker characteristics, as shown in Table 5. The high percentage of polymorphism (75%) and an average of 8.6 bands per primer are clear indications of the high genetic variability existing among the seven genotypes under investigation. This gives a solid foundation for further cluster analysis and construction of UPGMA dendrograms. Genetic Similarity Coefficient (Jaccard's). Dendrogram showing (Figure 3) the genetic relationship between seven genotypes of cereal crops based on RAPD markers using UPGMA Cluster Analysis. The horizontal axis represents the Jaccard similarity coefficient.

Cluster I (Triticum group): Qırmızı buğda and Yeni birlik form a high similarity node at 0.82, with a branching point at 0.70 for Yeni hibrid.

Cluster II (Hordeum and Triticale): The barley varieties (Multi-rowed and Murov) form a strong sub-cluster at 0.75 similarity.

The triticale varieties (Sevinc and Qismət) form a cluster, indicating their intermediate evolutionary position between the wheat and barley clusters.

CONCLUSION

Using RAPD (molecular storage) carrying a reference frame of molecular storage, data from this research demonstrates the degree of genetic variability amongst the population of Azerbaijani cereal species. The development of unique molecular ID profiles provides assurance as to whether an accession is

genetically identical or not and thus will provide justification for their use in marker-assisted selection (MAS) in breeding endeavors to achieve improved yield, as well as genetically pure cereals.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Morpho-agronomic characteristics and variability of selected cereal genotypes

No	Genotype	Plant height (cm)	Spike lentgh (cm)	Spike density (per 10 cm)	1000 grain weight (gm)	Yield potential (q/ha)
1	Qırmızıbuğda	95–105	8.5–10.0	18–20	38–42	40–50
2	Yeni birlik	90–100	9.0–11.0	20–22	40–44	45–55
3	Yeni hibrid	85–95	7.0–9.0	24–26	48–52	35–45
4	Multi-rowed	80–90	7.5–10.0	14–16	35–40	50–60
5	Murov	75–85	6.5–8.5	18–21	42–46	40–55
6	Sevinc	110–125	12.0-15.0	16–19	45–50	60–75
7	Gismet	105–120	11.0–14.0	17–20	44–48	55–70
	Mean	98.57	98.57	98.57	98.57	98.57
	C.D. (< 0.05)	5.24	0.92	1.15	2.36	4.85

Table 2: Characteristics of the RAPD primers and details of the studied cereal genotypes.

No	Genotype / Cultivar	Primer code	Nucleotide sequence(5' to 3')	Annealing temperature (Tm, °C)	GC content (%)
1	Qırmızı buğda	OPR-01	GACCTAGTGG	34.0	60
2	Yeni birlik	OPR-02	ACTGGCCTGA	34.0	60
3	Yeni hibrid	OPR-03	CCCGTTGCCT	32.0	70
4	Multi-row	OPR-04	TGAGCACGAG	34.0	60
5	Arpa – Murov	OPR-05	CCATTCCCCA	32.0	60
6	Tritikale – Sevinc	OPR-06	GTCTACGGCA	32.0	60
7	Tritikale – Gismet	OPR-07	CCCGTAGCAC	32.0	70

Table 3. Statistical characteristics and polymorphism levels of RAPD-PCR markers used for cereal genotype characterization

Marker parameter	Value
Total Number of Bands (TNB)	52
Number of Polymorphic Bands (NPB)	39
Number of Monomorphic Bands (NMB)	13
Percentage of Polymorphism (PPB, %)	75%
Average Bands per Primer	8.6
Size Range of Fragments (bp)	300 – 1500

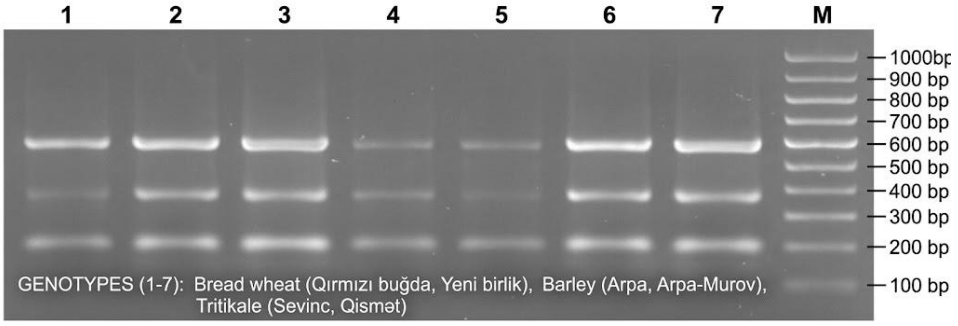

Figure 1. Representative RAPD-PCR profile using primer OPR-01 across seven studied cereal genotypes. Lane M: 100bp DNA ladder. Lanes 1-7: Cereal genotypes (e.g., Qırmızı buğda, Yeni birlik, Yeni hibrid, Arpa (Çoxcərgəli).

Figure 1: Electrophoretic profile of genomic DNA amplified with RAPD primer (OPR-01) in seven cereal genotypes. M: 100 bp DNA Ladder; Lanes 1–7: Investigated genotypes (1: Qırmızı buğda, 2: Yeni birlik, 3: Yeni hibrid, 4: Multi-rowed, 5: Murov, 6: Sevinc, 7: Gismet)

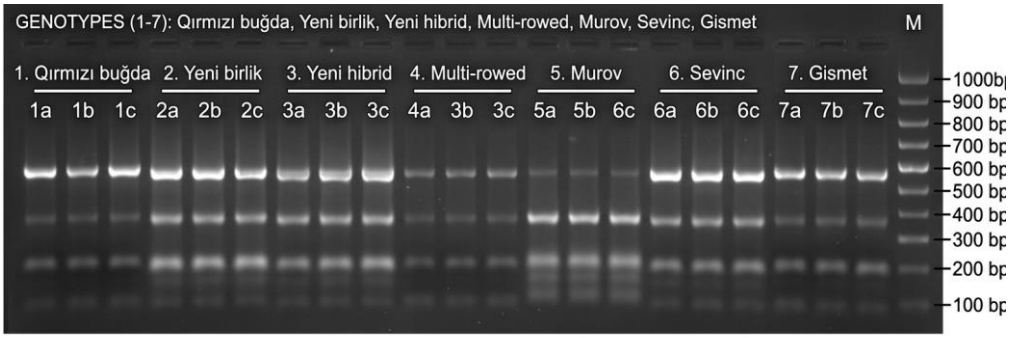

Figure 2. Representative RAPD-PCR profile using primer OPR-01 across seven studied cereal genotypes with three biological replicates each (total n=21). Lane M: 100 bp DNA ladder. Genotypes 1-7: Qırmızı buğda, Yeni birlik, Yeni hibrid, Multi-rowed, Murov, Sevinc Gismet. Lanes a-c for each genotype are biological replicates.

Figure 2. Representative RAPD-PCR profile using primer OPR-01 across seven studied cereal genotypes (n=21). Lane M: 100 bp DNA ladder. Lanes 1–7: Cereal genotypes (1: Girmizi bugda, 2: Yeni birlik, 3: Yeni hibrid, 4: Multi-rowed, 5: Murov, 6: Sevinj, 7: Gismet).

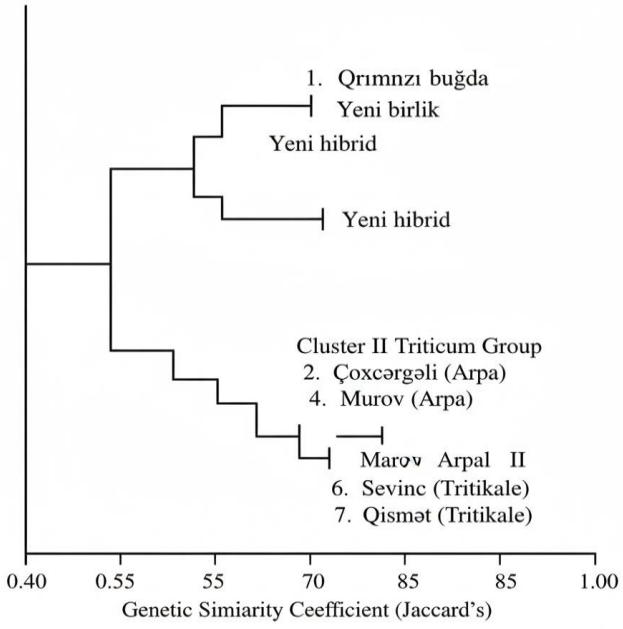

Figure 3: UPGMA dendrogram depicting the genetic relationships among seven cereal genotypes based on RAPD markers.