



Evaluation of genetic diversity using SSR markers and link with drought response of Azerbaijani durum wheat (*Triticum durum* Desf.) genotypes

Ruhangiz Mammadova · Ahmed Amri · Zeynal Akparov · Fida Alo · Fatma Sheikhzamanova · Mehraj Abbasov · Nurlan Amrahov · Elchin Hajiyev · Shader Alizade

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Abstract Genetic diversity of 45 genotypes were shown in the list of genotypes Azerbaijani durum wheat (*Triticum durum* Desf.) genotypes were screened using simple sequence repeats (SSRs). These accessions were collected from various bioclimatic regions of Azerbaijan. Out of the used 22 primers, 13 primers showed polymorphism and were selected for the analyses. Among the genotypes under study, 31 alleles were detected. The highest number of alleles was detected in locus gwm 335 (on chromosome 5B) and on locus gwm 445 (on chromosome

2A) with 5 and 4 alleles, respectively. The lowest number of alleles was found in locus gwm 617 with only 1 allele. For A, B, and D genomes, the total number of alleles detected was 14, 15, and 2, respectively. PIC value between studied SSR markers was 0.912 and this result shows high genetic diversity between Azerbaijani durum wheat genotypes. Therefore, these primers can be recommended for studying the genetic diversity of Azerbaijani durum wheat accessions. The genetic structure of the genotypes was analyzed and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram revealed five major clusters using Nei genetic distance index. The results revealed that SSR markers can efficiently evaluate genetic variation in the wheat samples. Based on the previous characterization of the drought response of these genotypes, links could be established between the SSR markers and drought tolerance. If some of the SSR markers are confirmed for their association with drought tolerance, then, they can be used as markers for the identification of drought-tolerant cultivars needed to enhance wheat productivity for farmers dealing with harsh conditions.

R. Mammadova (✉) · Z. Akparov · F. Sheikhzamanova · M. Abbasov · E. Hajiyev · S. Alizade
Genetic Resources Institute of the Ministry of Science and Education Republic of Azerbaijan, Baku, Azerbaijan
e-mail: ruhangiz.mammadova@fulbrightmail.org

R. Mammadova
Western Caspian University, Baku, Azerbaijan

R. Mammadova
The Department of Life Sciences, Khazar University, Baku, Azerbaijan

A. Amri
International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco

F. Alo
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, Andhra Pradesh, India

N. Amrahov · S. Alizade
Baku State University, Baku, Azerbaijan

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Introduction

Durum wheat is a high-value cereal crop primarily used for pasta production. Expansion of durum wheat crop production is currently limited, largely due to many biotic and abiotic stress factors, including heat, drought, mineral deficiency, soil salinity etc. Drought is one of the major threats to agricultural crop production including durum wheat in dry land. Plant growth, development, and yield are markedly reduced by the adverse effects of water deficiency. The growth reduction of durum wheat is also believed to be caused by impairing the physiological activities of mitochondria (Flagella et al. 2006). Natural genetic variation exists for drought tolerance within tetraploid wheat species which could be exploited for the improvement of drought tolerance (Munnis and Richards 2007). Breeding for drought tolerance includes screening of wheat germplasm to identify superior genotypes that could be used for the improvement of existing cultivars. The wild relatives and landraces offer the potential of additional sources of drought tolerance due to their adaptation to a wide range of environments and their rich genetic diversity (Colmer et al. 2006).

Conventional breeding relying on phenotypic selection is limited in its ability to accurately and effectively select for drought tolerance due to the complex nature of the trait. Molecular techniques such as molecular markers can be used to reveal sites of variation in DNA between individuals (Langridge and Chalmers 2005). A number of molecular marker technologies are routinely used in generating genetic linkage maps for both tetraploid and hexaploid wheat such as, simple sequence repeats (SSRs) (Roder et al. 1998). SSRs are short tandem repeats of nucleotide units (2–5 bp) that are codominant, abundant, and well dispersed in the genome (Lapitanz 1992; Tautz and Schlotterer 1994). SSR polymorphism can be detected due to variation in the number of these repeat units between the DNA samples. High-throughput detection methods have been established including Multiplex Ready Technology (MRT) (Hayden et al. 2008), which enables simultaneous detection of multiple SSR loci in an economical and efficient manner. To raise the density of microsatellite markers available on a wheat genetic map, Somers et al. (2004) constructed a high-density microsatellite consensus map by mapping common microsatellites on each chromosome in 4 different mapping populations.

Microsatellites can be exchanged between laboratories, and are highly transferable between populations (Song et al. 2005; Sun et al. 1998, 2003).

The assessment of genetic diversity is an essential component in the characterization and conservation of germplasm. Within the collections, some of the resources may be genetically redundant or duplicated and therefore not of significance. As conservation and maintenance of the germplasm is an expensive exercise, it is very important to accurately characterise and catalogue germplasm material in Gene bank. Information system of the Plant Genetic Resources (PGR) of Azerbaijan Gene bank plays an important role in the management of genetic resources and in the ongoing activities of the national PGR Network.

Genetic diversity in cultivated wheat has been narrowed due to extensive modern breeding approaches using a limited genetic base. In wheat landraces, diversity has been lost by the reduction in population size. To assist with enlarging the genetic base in breeding and conservation, it is important to assess the diversity present within these wheat landraces. The national breeding program, meanwhile, developed over 100 wheat cultivars, most of which had a significant impact on the Azerbaijan economy. Information on germplasm diversity and genetic relationships among cultivars are critical in wheat improvement. Genetic similarities might be evaluated using pedigree analysis (Barrett et al. 1998; Manifesto et al. 2005) or by assessing morphological traits (Schut et al. 1997) as well as biochemical (Metakovsky and Branlard 1998; Blanco et al. 2012) or, more recently, DNA markers (Plaschke et al. 1995; Roy et al. 2002; Burkhamer et al. 1997; Oman et al. 2001; Ogbonnaya et al. 2006; Rizza et al. 2012).

Germplasm characterization in the Gene bank of Genetic Resources Institute of Azerbaijan has been based mostly on morphological descriptors. However, the use of morphological descriptors, presents some limitations, such as limited polymorphism which would require a high number of descriptors, and potential environmental influence on the phenotype. Limited germplasm characterization of plant accessions conserved in Gene bank is a major cause for the limited use of accessions in breeding programs. High throughput genotyping of wheat germplasm accessions allows examination of genetic relationships and sampling of core collections representatives and reveals allelic richness of the Gene bank accessions.

So far, the genetic diversity of Azerbaijan-grown wheat cultivars are not completely studied with genetic markers. The objectives of this work were therefore to (i) characterize by SSR markers durum wheat cultivars, landraces, and cultivars grown in Azerbaijan; (ii) determine the existence of cultivar-specific amplicons; and (iii) assess the relative informativeness of SSRs.

Materials and methods

Plant material

A total of 45 durum wheat genotypes including drought-tolerant, semi-tolerant, and non-tolerant cultivars were used for molecular analysis. All used genotypes are tetraploids (*T. durum* ssp. *durum*) and include local cultivars and accessions collected during scientific expeditions. The name/pedigree of cultivars and all information about the genotypes used are presented in Table 1.

Microsatellite assay

A total of 22 wheat microsatellite (SSR) primers originated from two different groups were screened for polymorphism: Gwm primers from Institut für Pflanzengenetik und Kulturpflanzenforschung (Roder et al. 1997; Pestsova et al. 2000) (Table 2).

DNA extraction and PCR amplification

10 seeds of each genotype were planted in the greenhouse of the Biotechnology laboratory of ICARDA with a mean temperature of 18 °C. After ten days 5–7 cm long piece of fresh leaf material from each plant was cut and the leaf tissues were freeze-dried. Genomic DNA was extracted from fresh leaves with the PVP extraction protocol (F. Ogbonaya group 2006).

Ten µl of the isolated DNA and 2 µl of 10 × loading dye were loaded in a lane of 1.5% (w/v) agarose gel containing 0.05 µg ml⁻¹ ethidium bromide, for checking the quality of DNA.

For quantitative measurements, a charge-coupled device camera imaging system UVI soft analysis, and

Quantity one software were used to capture the image and to calculate the band intensities.

DNA quantification was studied as well with comparison of lambda DNA (10 ng/µl) and different dilution type of DNA (10 µl DNA:2 µl loading dye; 1/2DNA:2 µl loading dye; 1/4DNA:2 µl loading dye; 1/5DNA:2 µl loading dye).

PCR reaction mix was prepared corresponding to the protocol: 4.8 µl H₂O, 1 µl 10× buffer, 1 µl dntps, 0.5 µl Primer F and 0.5 µl Primer R, 0.1 µl Taq, and 2 µl DNA per reaction.

Amplifications were performed for each primer pair separately in microtiter plates using the following programs: 5 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 1 min annealing (between 50 and 62 °C, depending on the published optimal annealing temperature of the primer), and 30 s at 72 °C, and a final extension of 10 min at 72 °C.

Gel documentation and Data analysis

PCR products were separated on 8% PAGE gel, stained in ethidium bromide and visualized, UVI soft analysis was used to capture the image and to calculate the band intensities. Cluster analysis demonstrating the genetic relationship between genotypes was generated using a simple matching coefficient and Unweighted Pair Group Method Using Arithmetic Averages (UPGMA) and *Nei* genetic distance. The results are presented graphically in dendrograms.

After gel documentation, results were scored for the construction of cluster analysis using the Power Marker software program.

Polymorphism informative content (PIC)—PIC value is one of the basic steps in determining genetic diversity of genotypes by markers and helps to distinguish molecular differences. The PIC concept is used to measure the effectiveness of a marker and its informativeness in a given population. PIC value was calculated using formula:

$$PIC = 1 - \sum p_{ij}^2$$

where P_{ij} is the frequency of the j th allele.

Table 1 Pedigree of genotypes of durum wheat used for molecular tests

N	CN	Subtaxa	Population type	Collection site
1	6085	var. <i>leucurum</i>	LA	Akstafa
2	Ter-ter	var. <i>provinciale</i>	CV	–
3	6086	var. <i>leucurum</i>	LA	Gazakh
4	6087	var. <i>leucurum</i>	LA	Aghdara
5	6089	var. <i>leucurum</i>	UM	–
6	Ag bugda		CV	–
7	Barakatli 95	var. <i>hordeiforme</i>	CV	–
8	Shiraslan	var. <i>leucurum</i>	CV	–
9	6090	var. <i>leucurum</i>	LA	Terter
10	6091	var. <i>leucurum</i>	LA	Khachmaz
11	6092	var. <i>leucurum</i>	LA	Aghdam
12	6093	var. <i>leucurum</i>	LA	Goychay
13	6094	var. <i>leucurum</i>	LA	Aghsu
14	6095	var. <i>leucurum</i>	UM	–
15	6096	var. <i>leucurum</i>	UM	–
16	6097	var. <i>leucurum</i>	UM	–
17	shirvan	var. <i>hordeiforme</i>	CV	–
18	Vugar	var. <i>leucurum</i>	CV	–
19	6098	var. <i>leucurum</i>	LA	Aghdash
20	Mugan	var. <i>leucomelan</i>	CV	–
21	6099	var. <i>leucurum</i>	LA	Khanlar
22	6100	var. <i>leucurum</i>	LA	Barda
23	6102	var. <i>leucurum</i>	LA	Shamakhi
24	Mirbashir 50	var. <i>leucurum</i>	CV	–
25	6103	var. <i>hordeiforme</i>	LA	Shaki
26	6104	var. <i>hordeiforme</i>	LA	Terter
27	6105	var. <i>hordeiforme</i>	UM	–
28	Gara gilchiq 2	var. <i>apulicum</i>	CV	–
29	6106	var. <i>hordeiforme</i>	UM	–
30	6107	var. <i>hordeiforme</i>	LA	Shamakhi
31	6108	var. <i>hordeiforme</i>	LA	Barda
32	6109	var. <i>hordeiforme</i>	LA	Aghdam
33	6110	var. <i>hordeiforme</i>	LA	Mingechevir
34	6111	var. <i>hordeiforme</i>	LA	Akstafa
35	6112	var. <i>hordeiforme</i>	LA	Shamkir
36	6113	var. <i>hordeiforme</i>	LA	Gakh
37	6114	var. <i>hordeiforme</i>	LA	Yevlakh
38	6115	var. <i>hordeiforme</i>	LA	Nakhchivan
39	6116	var. <i>hordeiforme</i>	LA	Nakhchivan
40	6118	var. <i>hordeiforme</i>	LA	Nakhchivan
41	Sharg	var. <i>leucurum</i>	CV	–
42	6120	var. <i>hordeiforme</i>	LA	Nakhchivan
43	6121	var. <i>boeufii</i>	LA	Shamakhi
44	6122	var. <i>boeufii</i>	LA	Masalli
45	6125	var. <i>melanopus</i>	LA	Mingechevir

CN catalogue number,
 RM research material,
 CV cultivar, UM unrelised
 breeder material, LA land
 race

Table 2 Microsatellite markers, their expected size and sequence

SSR marker	Expected size, bases	Forward	Reverse
gwm Gwm 257		AGAGTGCATGGTGGGACG	CCAAGACGATGCTGAAGTCA
gwm Gwm 311	120–143–157	TCACGTGGAAGACGCTCC	CTACCTGCACCACCACCATTTT
gwm 335	203–240	CGTACTCCACTCCACACGG	CGGTCCAAGTGCTACCTTTC
gwm 357		TATGGTCAAAGTTGGACCTCC	AGGCTGCAGCTCTTCTTCAG
gwm 368		TATGGTCAAAGTTGGACCTCG	AGGCTGCAGCTCTTCTTCAG
gwm 369		CTGCAGGCCATGATGATG	ACCGTGGGTGTTGTGAGC
gwm 408	148–182	TCGATTTATTTGGGCCACTG	GTATAATTCGTTTCACAGCACGC
gwm 44	176–178	GTTGAGCTTTTCAGTTCGGC	ACTGGCATCCACTGAGCTG
gwm 471	130	CGGCCCTATCATGGCTG	GTTTGCAAGTTCATTTTGC
gwm 493		TTCCATAACTAAAACCGCC	GGAACATCATTTCTGGACTTTG
gwm 494		ATTGAACAGGAAGACATCAGG	TTCCTGGAGCTGTCTGGG
gwm 518	154–166	AATCACAACAACAAGGCGTGAA	CAGGGTGGTGCATGCAT
gwm 537	207	ACATAATGCTTCCTGTGCACC	GCCACTTTTGTGTCGTTTCT
gwm 6		CGTATCACCTCCTAGCTAAACTAG	AGCCTTATCATGACCCTACCTT
gwm 610		CTGCCTTCTCCATGGTTTGT	AATGGCCAAAGGTTARGAAGG
gwm 617	133–154–164	GATCTTGGCGCTGAGAGAGA	CTCCGATGGATTACTCGCAA
gwm 666	114	GCACCCACATCTTCGACC	TGCTGCTGGTCTCTGTGC

Results and discussion

The research aimed to investigate the genetic polymorphism of a mapping population of durum wheat obtained from Gene bank accessions of The Genetic Resources Institute of the Ministry of Science and Education and included land races, research material, unreleased breeder material, and local cultivars. To assess genetic distance among 45 durum wheat genotypes 17 microsatellite markers were used out of which 13 makers were polymorphic. Markers gwm 6, gwm 368, gwm 369, gwm 518 didn't show polymorphism in our experiments. The number of alleles per locus ranged from 1 (gwm 617) to 5 (gwm 335) (Table 3).

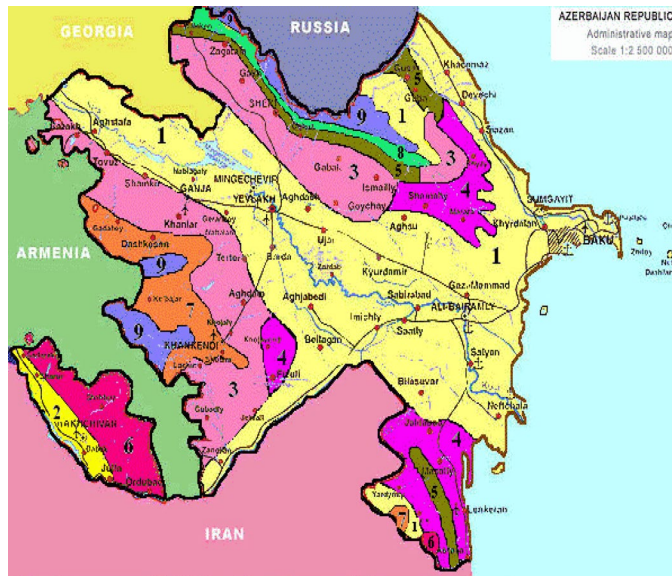
Collection Sites of Genebank accessions and Climatic zones of Azerbaijan

Seed material was collected by expeditions from different regions of Azerbaijan. To analyze the relationship between cluster groups we need to collect information about collection sites. As it is known Azerbaijan has multiform climatic conditions, 9 out

Table 3 Microsatellite markers, the amplified number of alleles, their chromosomal location, and polymorphism information content in a sample of Azerbaijani durum wheat accessions

Mn	Na	Cl	PIC value
gwm 493	2	3B	0.99
gwm 357	2	1A	0.98
gwm 494	2	6A	0.87
gwm 610	3	4A	0.94
gwm 617	1	2A	0.72
gwm 335	5	5B	0.94
gwm 537	2	7B	0.98
gwm 257	2	2B	0.98
gwm 408	2	5B	0.99
gwm 44	2	7D	0.83
gwm 445	4	2A	0.73
gwm 311	2	2B	0.94
gwm 471	2	7A	0.97
Mean			0.912

Mn marker name, *Na* number of alleles, *Cl* chromosomal location of a marker, *PIC* polymorphism information content



- 1 half- deserts and dry steppes with soft winter and dry hot climate
- 2 half- deserts and dry steppes with cold winter and dry hot climate
- 3 moderately warm climate with dry winter
- 4 moderately warm climate with dry summer
- 5 moderately warm climate almost with uniform distribution of sedimentary
- 6 cold climate with dry summer
- 7 cold climate with dry winter
- 8 cold climate with plentiful quantity of sedimentary in all seasons
- 9 climate mountainous tundra's

Fig. 1 Climatic patterns of Azerbaijan

of the 11 climate patterns in the Koppel climate classification can be found in Azerbaijan (Fig. 1).

Drought response of genotypes are presented in Table 4 and are derived from the Catalogue of Azerbaijan Research Institute of Crop Husbandry (2013).

Polymorphism analysis of durum wheat accessions

The dendrogram is based on the similarity of genotypes and divides the genotypes into five large groups. The first group is homogenous and consists of genotypes (6112, 6108, and 6104), all belonging to the *var. hordeiforme* subtaxa and collected from the same collection site 1 which has a dry climate - half deserts and dry steppes with soft winter.

Table 4 Drought response of some tested durum wheat cultivars

No. genotype	Cultivar	Drought response
1	Mirbashir-50	Highly tolerant
2	Shiraslan-23	Tolerant
3	Barakatli-95	Tolerant
4	Shirvan	Moderately tolerant
5	Sharq	Moderately tolerant
6	Tertter	Moderately tolerant
7	Garagilchig-2	Highly tolerant
8	Vugar	Moderately tolerant
9	Mugan	Not tolerant

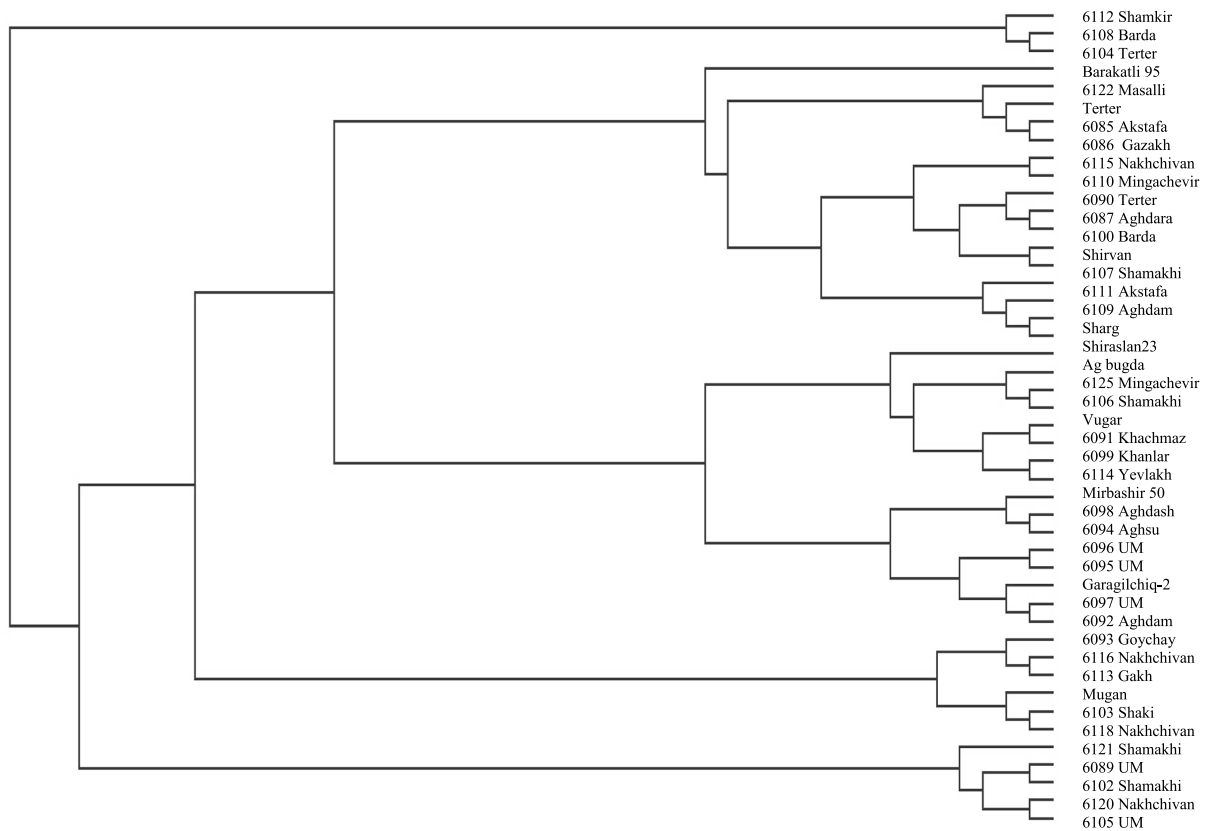


Fig. 2 Dendrogram of 45 durum wheat *Triticum durum* Desf. genotypes using SSR markers

The second group consists of 16 genotypes combining Barakatli-95, Terter, Shirvan, Sharg and, Shiraslan cultivars and 11 land races. Barakatli-95 stood apart from other subgroups but the common feature for all cultivars is their drought response. As shown in Table 2, all cultivars of this cluster group are moderately tolerant to drought tolerant including the cultivar Barakatli-95 (Fig. 2).

The third group can be divided into sub-clusters totaling 16 genotypes: Ag bugda, Vugar, Mirbashir-50, and Garagilchiq-2 cultivars, genotypes 6096, 6097 and 6095 (unreleased research material) and 9 land races. This group included both highly tolerant cultivars Mirbashir-50 and Garagilchiq-2 and moderately tolerant Vugar. Research germplasm having similar electrophoresis profile with drought tolerant cultivars and therefore can be characterized as genotypes with high drought tolerance. The land races of the third group were collected from collection sites 1 and 3 (half deserts and dry steppes with cold winter and dry hot climate). High genetic similarity

between genotypes in this group can be related to the climate features of their collection sites. Most of the genotypes originated from collection site-1 and site-3. These two collection sites are geographically distant but belong to the same climate classification zone with moderately warm climate and dry winter. The 4th cluster group consists of five land races and the cultivar Mugan which has low drought tolerance. Land races of this cluster group are polymorphic according to their collection sites as collected from sites 3, 4, and 5, all characterized for their moderately warm climate with dry summer. These sites are in between of sites with dry hot climates (sites 1 and 2) and sites with cold climates (5, 6, 7, 8). This can explain the genetic similarity of land races from these sites and the cultivar Mugan. The 5th group is combining research material 6089 and 6105 and three landraces collected from sites 1 and 2.

This study determined the genetic diversity among 45 durum wheat genotypes. The thirteen markers amplified a total 31 alleles. The highest numbers of

alleles were detected for locus gwm 335 (on chromosome 5B) and locus gwm 445 (on chromosome 2A) with 5 and 4 alleles respectively. The lowest number of alleles was determined in locus gwm 617 with only 1 allele. For A, B, and D genomes, the total number of alleles detected was 14, 15, and 2, respectively.

The PIC value between studied SSR markers was 0.912 and shows high genetic diversity between studied durum wheat genotypes. Therefore, these primers can be recommended for the genetic evaluation of Azerbaijan wheat accessions. Khavarinejad and Karimov (2012) detected 33 and 17 polymorphic alleles for spring wheat genotypes by RAPD and SSR markers, respectively. Xgwm 120-2B had the most PIC with 57%. The obtained results showed an average of 3.4 polymorphic bands per primer for SSR.

Research conducted to assess in formativeness and efficiency of 26 different molecular markers for genetic diversity among 40 durum wheat determined 74% of the diversity. A high level of polymorphism was recorded for SSR markers used in the study. A total of 136 fragments were obtained from the 26 SSR primers and all the bands were polymorphic across all the genotypes screened. The PIC values ranged from 38 to 94% with an average of 74% (Ratiba et al. 2012). In the study conducted by Khavarnejad (2013) polymorphism percentage was detected by UBC 350 and UBC 109 markers with values 0.53 and 0.50 respectively using RAPD and concluded that RAPD could detect more polymorphism alleles than SSRs.

In our experiments more differentiations were observed between accessions in populations originating from different climatic regions of Azerbaijan. Similar results were observed in experiments conducted to study the genetic diversity of Ethiopian durum wheat landrace collections. The genetic diversity of 141 Ethiopian durum wheat landrace collections and 19 cultivars was analyzed using 12 SSR primers. Based on the analysis of molecular variance, a lower level (19%) of variation was observed among populations and a higher level (81%) within the population (Asmamaw et al. 2019).

Breeding programs focused on morphological traits also can affect diversity among wheat accessions. morphological characterization and genetic diversity analysis of Tunisian durum wheat accessions revealed high genetic variation within regions (81%) and within genetic groups (41%), reflecting a considerable amount of admixture between landraces.

The moderate (19%) and high (59%) levels of genetic variation detected among regions and genetic groups, respectively, highlighted the selection practices of local farmers (Ouaja et al. 2021).

Christov et al. (2022) studied of population structure in combination with genetic variation has big importance for the improvement of breeding programs under drought-prone environmental conditions. The genetic variation and population structure of a panel of 90 durum wheat consisting of 62 cultivars and breeding lines originating from two agro-ecological zones in Bulgaria (Northern and Southern Bulgaria) and 28 introduced cultivars from Southwestern, Central, and Eastern Europe, and the USA showed a good relationship between the genetic and phenotypic population structures according to the division of the genotypes by their agro-geographical origin.

Dagnaw et al. (2023) studied 104 Ethiopian durum wheat genotypes representing thirteen populations, three regions, and four altitudinal classes for their genetic diversity, using 10 grain quality- and grain yield-related phenotypic traits and 14 simple sequence repeat (SSR) makers. Analysis of molecular variance (AMOVA) revealed a high level of variation within populations, regions, and altitudinal classes, accounting for 88%, 97%, and 97% of the total variation, respectively.

Summarizing the characterization results of 45 durum wheat genotypes we conclude that genotypes having similar profiles with drought-tolerant cultivars Mirbashir-50 and Garagilchig-2 can be characterized as genotypes with high tolerance to drought. Most of the land races of this cluster group were collected from half deserts and dry steppes prevailing in the collection site 1, which could explain the importance of natural selection of landraces and their adaptation to drought. Genetic similarity of genotypes can be explained by the presence of common alleles probably related to drought resistance. More SSR markers will be needed to further confirm the tolerance to drought of the genotypes in this group.

The research findings may be useful for the improvement of drought tolerance in durum wheat crop cultivars.

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Author contributions All authors contributed to the study conception and design. Material preparation was performed by ZA and NA, phenotypic data collection was performed by FS, EH, and MA, experiment design and molecular analysis was performed by RM, FYA and AA. The first draft of the manuscript was written by RM and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets analyzed during the current study are available from the corresponding author – Ruhangiz Mammadova upon request.

Declarations

Conflict of interest Financial interests: author Ruhangiz Mammadova, Ahmed Amri, Zeynal Akparov, Fida Yossef Alo, Fatma Shekhzamanova, Mehraj Abbasov, Nurlan Amrahov, Elchin Hajiyev, Shader Alizade declare they have no financial interests. Author Ruhangiz Mammadova received training and travel support from the Japan international cooperation agency (JICA) in 2011. The experimental part of the work was implemented during the outlined long-term training program.

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