Evaluation of Genetic Diversity Using Ssr Markers and Link With Drought Response of Azerbaijani Durum Wheat (Triticum Durum L.) Genotypes

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

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Abstract: Genetic diversity of 46 Azerbaijani durum wheat (*Triticum durum* L.) genotypes were screened using simple sequence repeats (SSRs). These accessions were collected from various bioclimatic regions of Azerbaijan. Out of the used twenty-two primers, 13 primers showed polymorphism and were selected for the analyses. Among the genotypes under study, 31 alleles were detected. 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 determined in locus Gwm 617 with only 1 allele. For A, B and D genomes, the total number of alleles detected were 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 genetic diversity of Azerbaijani durum wheat accessions. The genetic structure of the genotypes was analyzed and the 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 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 s with drought tolerance, then, they can be used as markers for identification of drought tolerant varieties needed to enhance wheat productivity for farmers dealing with harsh conditions.

Key words: durum, wheat, drought, stress, SSR markers, genetic diversity, polymorphism

1. İntroduction

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 for agricultural crop production including durum wheat in dry land. Plant growth, development and yield are markedly reduced by the adverse effects of water deficiency. 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 which could be used for 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 (2-5bp) that are codominant, abundant and well dispersed in genome (Lapitanz 1992; Tautz and Schlotterer 1994). SSR polymorphism can be detected due to variation in 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 economic 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 assesment 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 Genbank. Information system of the PGR of Azerbaijan Genebank which plays an important role for the management of genetic resources and in on-going activities of the national PGR Network.

Genetic diversity in cultivated wheat has been narrowed due to extensive modern breeding approaches using a narrow 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 by means of pedigree analysis (Barrett et al. 1998,

89 Manifesto et al. 2005) or by assessing morphological traits (Schut et al. 1997) as well as biochemical
90 (Metakovsky and Branlard 1998, Blanco et al. 2012) or, more recently, DNA markers (Plaschke et al.
91 1995, Roy et al. 2002, Burkhamer et al. 1997, Oman et al. 2001, Ogbonnaya et al. 2006, Rizza et al.
92 2012).
93 Germplasm characterization in Genebank of Genetic Resources Institute of Azerbaijan has been based
94 mostly on morphological descriptors. However, the use of morphological descriptors, present some
95 limitations, such as limited polymorphism which would require a high number of descriptors, potential
96 environmental influence on the phenotype. Limited germplasm characterization of plant accessions
97 conserved in Genebank is a major cause for the limited use of accessions in breeding programs. High
98 throughput genotyping of wheat germplasm accessions allows examination of genetic relationships and
99 sampling of core collections representatives and reveal allelic richness of the Genebank accessions.
100 So far, the genetic diversity of Azerbaijan-grown wheat cultivars are not competely studied with
101 genetic markers. The objectives of this work were therefore to i) characterize by SSR markers durum
102 wheat cultivars, landraces and varieties grown in Azerbaijan; ii) to determine the existence of cultivar-
103 specific amplicons; and iii) to assess the relative informativeness of SSRs.

104 **2. Materials and Methods**

105 **Plant material**: A total of 45 durum wheat genotypes including drought tolerant, semi-tolerant and non-
106 tolerant varieties were used for molecular analysis. All used genotypes are tetraploids (*T. durum* ssp.
107 *durum*) and include local cultivars and accesions collected during scientific expeditions. Name/pedigree
108 of cultivars and all information about genotypes used are presented in Table 1.

109 **Table 1**. Pedigree of genotypes of durum wheat used for molecular tests
110

N	CN	Subtaxa	Population type	Collection site
1	6085	*var. leucurum*	LA	Akstafa
2	Ter-ter	*var.provinciale*	CV	-
3	6086	*var. leucurum*	LA	Gazakh
4	6087	*var. leucurum*	LA	Aghdara
5	6089	*var. leucurum*	UM	-
6	Ag bugda		CV	-
7	Barakatli 95	*var.hordeiforme*	CV	-
8	Shiraslan	*var.leucurum*	CV	-
9	6090	*var. leucurum*	LA	Terter
10	6091	*var. leucurum*	LA	Khachmaz
11	6092	*var. leucurum*	LA	Aghdam

12	6093	*var. leucurum*	LA	Goychay
13	6094	*var. leucurum*	LA	Aghsu
14	6095	*var. leucurum*	UM	-
15	6096	*var. leucurum*	UM	-
16	6097	*var. leucurum*	UM	-
17	Shirvan	*var.hordeiforme*	CV	-
18	Vugar	*var.leucurum*	CV	-
19	6098	*var. leucurum*	LA	Aghdash
20	Mugan	*var.leucomelan*	CV	-
21	6099	*var. leucurum*	LA	Khanlar
22	6100	*var. leucurum*	LA	Barda
23	6102	*var. leucurum*	LA	Shamakhi
24	Mirbashir 50	*var. leucurum*	CV	-
25	6103	*var. hordeiforme*	LA	Shaki
26	6104	*var. hordeiforme*	LA	Terter
27	6105	*var. hordeiforme*	UM	-
28	Gara gilchiq 2	*var.apulicum*	CV	-
29	6106	*var. hordeiforme*	UM	-
30	6107	*var. hordeiforme*	LA	Shamakhi
31	6108	*var. hordeiforme*	LA	Barda
32	6109	*var. hordeiforme*	LA	Aghdam
33	6110	*var. hordeiforme*	LA	Mingechevir
34	6111	*var. hordeiforme*	LA	Akstafa
35	6112	*var. hordeiforme*	LA	Shamkir
36	6113	*var. hordeiforme*	LA	Gakh
37	6114	*var. hordeiforme*	LA	Yevlakh
38	6115	*var. hordeiforme*	LA	Nakhchivan
39	6116	*var. hordeiforme*	LA	Nakhchivan
40	6118	*var. hordeiforme*	LA	Nakhchivan
41	Sharg	*var.leucurum*	CV	-
42	6120	*var. hordeiforme*	LA	Nakhchivan
43	6121	*var. boeufii*	LA	Shamakhi
44	6122	*var. boeufii*	LA	Masalli
45	6125	*var. melanopus*	LA	Mingechevir

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

2.1 Microsatellite assay

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

Table 2. Microsatellite markers, their expected size and sequence

SSR marker	Expected size, bases	Forward	Reverse
GWM257	-	AGAGTGCATGGTGGGACG	CCAAGACGATGCTGAAGTCA
GWM 311	120-143-157	TCACGTGGAAGACGCTCC	CTACCTGCACCACCACCATTTT
GWM 335	203-240	CGTACTCCACTCCACACGG	CGGTCCAAGTGCTACCTTTC
GWM357	-	TATGGTCAAAGTTGGACCTCC	AGGCTGCAGCTCTTCTTCAG
GWM368	-	TATGGTCAAAGTTGGACCTCG	AGGCTGCAGCTCTTCTTCAG
GWM369	-	CTGCAGGCCATGATGATG	ACCGTGGGTGTTGTGAGC
GWM408	148-182	TCGATTTATTTGGGCCACTG	GTATAATTCGTTCACAGCACGC
GWM 44	176-178	GTTGAGCTTTTCAGTTCGGC	ACTGGCATCCACTGAGCTG
GWM471	130	CGGCCCCTATCATGGCTG	GTTTGCAAGTTCCATTTTGC
GWM493	-	TTCCCATAACTAAAACCGCC	GGAACATCATTTCTGGACTTTG
GWM494	-	ATTGAACAGGAAGACATCAGG	TTCCTGGAGCTGTCTGGG
GWM518	154-166	AATCACAACAACAAGGCGTGAA	CAGGGTGGTGCATGCAT
GWM537	207	ACATAATGCTTCCTGTGCACC	GCCACTTTTGTGTCGTTCCT
GWM 6	-		
GWM610	-	CTGCCTTCTCCATGGTTTGT	AATGGCCAAAGGTTARGAAGG
GWM617	133-154-164	GATCTTGGCGCTGAGAGAGA	CTCCGATGGATTACTCGCAA
GWM666	114	GCACCCACATCTTCGACC	TGCTGCTGGTCTCTGTGC

DNA extraction -10 seeds of each genotype were planted in greenhouse of 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 PVP extraction protocol (F. Ogbonaya group 2006).

DNA quantification and gel documentation

Ten µl of the isolated DNA and 2 µl of 10 x loading dye was 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 and 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 (10ng/µl) and different dilution type of DNA (10 µl DNA:2µl loading dye; 1/2DNA:2µl loading dye; 1/4DNA:2µl loading dye; 1/5DNA:2µl loading dye).

PCR reaction mix was prepared corresponding to the protocol: 4,8 µl H2O, 1µl 10x buffer, 1µl dntps, 0.5µl Primer F and 0.5µl Primer R, 0,1µl Taq, and 2µl 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 sec at 94°C, 1 min annealing (between 50 and 62 °C, depending on the published optimal annealing temperature of the primer), and 30 sec at 72°C, and a final extension of 10 min at 72°C.

Gel documentation: PCR products were separated on 8% PAGE gel, stained in ethidium bromide and vizualized, UVI soft analysis were used to capture the image and to calculate the band intensities. Cluster analysis demonstrating genetic relationship between genotypes were generated using 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 construction of cluster analysis using 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. PIC concept is used to measure the effectiveness of a marker and its informativeness in a given population. PIC value was calculated using formula:

$PIC= 1-\Sigma pij^2$

Where Pij is the frequency of the j^{th} allele.

3. Results and discussion

The aim of the research was to investigate the genetic polymorphism of a mapping population of durum wheat obtained from Genebank accessions of Genetic Resources Institute of Azerbaijan National Academy of Sciences and included land races, research material, unreleased breeder material and local cultivars. To assess genetic distance among 46 durum wheat genotypes 17 microsatellite markers were used out of which 13 makers were polymorphic. Markers Gwm6, Gwm368, 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).

Table 3. Microsatellite markers, the amplified number 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

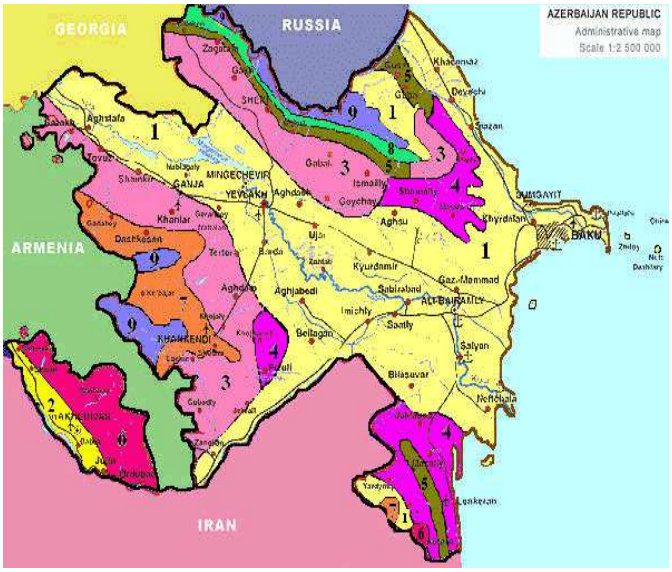
This study determined the genetic diversity among 45 durum wheat genotypes. The thirteen markers amplified a total 31 allels. The highest numbers of alleles was detected for locus gwm 335 (on chromosome 5B) and locus gwm 445 (on chromosome 2A) with 5 and 4 alleles respectively. The lowest number of alleles was determined in locus gwm 617 with only 1 allele. For A, B and D genomes, the total number of alleles detected were 14, 15 and 2, respectively.

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 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. High level of polymorphism was recorded for SSR markers used in 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 value 0.53 and 0.50 respectively using RAPD and concluded that RAPD could detect more polymorphism alleles than SSRs.

Collection Sites of Genebank accessions and Climatic zones of Azerbaijan.

Seed material was collected by expeditions from different regions of Azerbaijan. To analyze relationship between cluster groups we need to collect information about collection sites. As it is known Azerbaijan has multiform climatic conditions, 9 out of the 11 climate patterns in the Koppel climate classification can be found in Azerbaijan.



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

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

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	Terter	Moderately tolerant
7	Garagilchig-2	Highly tolerant
8	Vugar	Moderately tolerant
9	Mugan	Not tolerant

Polymorphism analysis of durum wheat accessions

Dendrogram is based on similarity of genotypes divided the genotypes into five large groups. The first group is homogenous and consisting of genotypes (6112, 6108 and 6104), all belonging to the *var.*

hordeiforme subtaxa and collected from the same collection site 1 which has dry climate - half deserts and dry steppes with soft winter.

The second group is consisting 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 it shown on table 2, all cultivars of this cluster group are moderately tolerant to drought tolerant including the cultivar Barakatli-95.

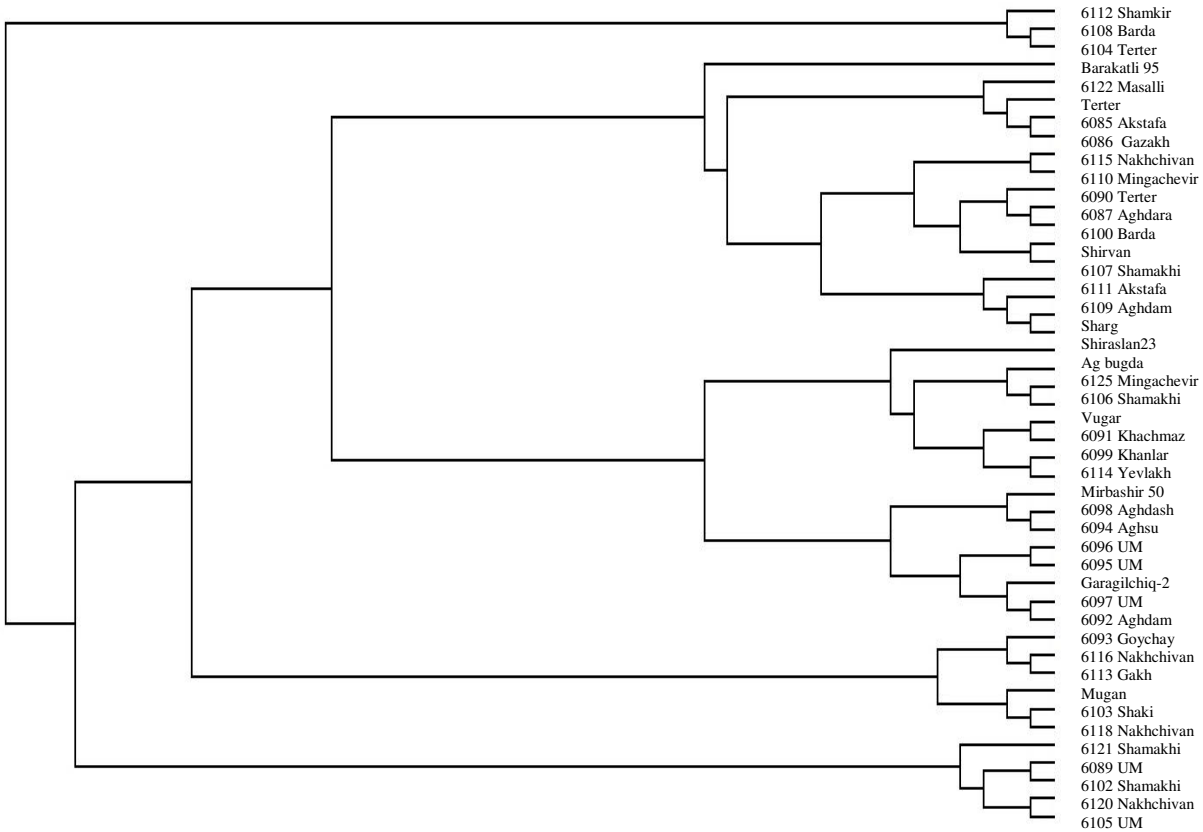


Fig. 2. Dendrogram of 45 durum wheat (*Triticum durum* L.) genotypes using SSR markers

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 Garagilchig-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 genotypes originated from collection site-1 and site-3. This 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 Mugam 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 climate (sites 1 and 2) and sites with cold climate (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.

Summarizing the characterization results of 45 durum wheat genotypes we conclude that genotypes having similar profile with drought tolerant cultivars Mirbashir-50 and Garagilchiq-2 can be characterized as genotypes with high tolerance to drought. Most of 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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Statements and declarations

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Competing interests

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 has received training and travel support from japan international cooperation agency (JICA) in 2011. Experimental part of work was implemented during outlined long term training program.

Author contributions

All authors contributed to the study conception and design. Material preparation was performed by Zeynal Akparov and Nurlan Amrahov, phenotypic data collection were performed by Fatma Sheikhzamanova, Elchin Hajiyev and Mehraj Abbasov, experiment design and molecular analysis were performed by Ruhangiz Mammadova, Fida Yossef Alo and Ahmed Amri. The first draft of the manuscript was written by Ruhangiz Mammadova and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability

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