

ASSESSMENT OF GENETIC POLYMORPHISM IN DURUM AND BREAD WHEAT ACCESSIONS OF AZERBAIJAN

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Abstract

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The present paper aims at analyzing the genetic diversity among 110 bread and 110 durum wheat accessions and local varieties using inter simple sequence repeat (ISSR) markers. Eight ISSR primers generated a total of 107 and 136 PCR fragments for durum and bread wheat genotypes, respectively. In average ISSR primers produced 17 bands for bread and 13.3 bands for durum wheat genotypes. The level of genetic diversity (GDI) was quite high for both wheat species and averaged 0.94 for durum and 0.91 for bread wheat accessions. Results of cluster analysis of hard and soft wheat enabled to allocate 11 and 12 main clusters, respectively and revealed the complex nature of the distribution of the studied varieties and botanical varieties. There was no particular relation between genetic structure of genotypes and their geographical distribution. The most remote genotypes identified as a result of cluster analysis can be recommended for use by breeders to produce genetically diverse populations with a maximum range of variation in the hybrid offspring.

Key words: ISSR markers; bread wheat; durum wheat; genetic diversity

Introduction

Wheat production in the world is mainly based on 2 species, bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* Desf.) (Kahrizi et al., 2010). In the light of the current expectations of population growth, world wheat production has to be massively increased to sustain the associated food demand rise. Because of the land limitations the enhancement of wheat production must come from higher yields, which can be met by increased efforts in plant breeding (Braun et al., 1998). Genetic diversity of wheat cultivars is very important in reducing genetic vulnerability during plant breeding efforts. Azerbaijan is one the centers of origin and

diversification of cereal species. Extreme diversity of the soil and climatic conditions of Azerbaijan support a very rich diversity of cereal plants, including wheat. Collection of wheat species, botanical varieties and populations has been successfully implemented in the country and wheat collection which contain more than 2 000 accessions has been created in National Gene Bank. Evaluation of diversity among these wheat cultivars and botanical varieties is essential due to the presence of genetic variability in well-adapted backgrounds.

Morphological and biochemical markers used in determination of genetic diversity may not adequately represent the genetic diversity due to low polymorphism, heritability and sensitivity to environmental conditions. Molecular genetic

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markers proved to be a valuable tool to assess the genetic diversity that is often masked by undesirable phenotypes (Sönmezoğlu et al., 2012). The commonly used polymerase chain reaction (PCR)-based DNA marker systems are random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs) and inter simple sequence repeats (ISSR). These techniques have been widely applied to evaluate genetic diversity among different plant, as well as wheat (Aliyev et al., 2007; Babayeva et al., 2009; Angelov et al., 2017). ISSR markers detect polymorphisms in inter-microsatellite loci using a single primer composed of a simple sequence repeat (SSR) sequence anchored at the 3' or 5' end by 2–4 arbitrary nucleotides. ISSR markers have the advantages of relatively low cost, high polymorphism and good reproducibility (Izzatullayeva et al., 2014; Tonk et al., 2014; Hajiyev et al., 2015; Saleh et al., 2017). The technique combines most of the benefits of AFLP and microsatellite analysis with the universality of RAPD (Reddy et al., 2002). The objective of

this study was to evaluate genetic diversity among bread and durum wheat accessions and botanical varieties using ISSR markers in order to determine the significance of the gene pool and the manner in which it can be utilized.

Materials and Methods

The plant material

The material for molecular-genetic studies were the 10 local varieties (Sharg, Tartar, Vugar, Aghbugda, Bereketli 95, Mirbashir50, Garagylchyg 2, Mugan, Shiraslan, Shirvan) and 100 accessions of durum wheat got from genebank of GRI, as well as 12 varieties (Ruzi 84, Mirbashir 128, Akinchi 84, Gobustan, Shaki 1, Gyrmzy bughda, Gunashly, Shafag, Azamatli 95, Yegana, Azeri and Parzivan 2) and 98 accessions of bread wheat belonged to different botanical varieties and collected from different regions of Azerbaijan. The names and origins of the studied genotypes were presented in Table 1 and Table 2.

Table 1

Gene Bank number, botanical varieties and geographical origin of durum wheat accessions used in the study

GeneBank number	Sample name	Geographical origination	GeneBank number	Sample name	Geographical origination
1	2	3	4	5	6
6152	var. <i>apulicum</i>	Nakhchivan	6144	var. <i>leucomelan</i>	Lerik
6101	var. <i>leucurum</i>	Tovuz	6138	var. <i>leucomelan</i>	Evlakh
6304	var. <i>leucurum</i>	Absheron	6130	var. <i>murciense</i>	Saatli
6109	var. <i>hordeiforme</i>	Agdam	6309	var. <i>leucomelan</i>	Absheron
6123	var. <i>melanopus</i>	Tovuz	6135	var. <i>leucomelan</i>	Agdzhabadi
6086	var. <i>leucurum</i>	Kazakh	6121	var. <i>boeufii</i> S	Shamakhi
6157	var. <i>apulicum</i>	Nakhchivan	6160	var. <i>reichenbachii</i>	Zakatala
6136	var. <i>leucomelan</i>	Devichi	6310	var. <i>leucomelan</i>	Absheron
6148	var. <i>apulicum</i>	Ismaili	6156	var. <i>apulicum</i>	Nakhichevan
6087	var. <i>leucurum</i>	Agdara	6314	var. <i>leucurum</i>	Absheron
6133	var. <i>alborovinciale</i>	Dzhalilabad	6119	var. <i>hordeiforme</i>	Nakhchivan
6154	var. <i>apulicum</i>	Nakhchivan	6128	var. <i>melanopus</i>	Kazakh
6158	var. <i>obscurum</i>	Zakatala	6124	var. <i>melanopus</i>	Geranboy
6089	var. <i>leucurum</i>	Evlakh	6098	var. <i>leucurum</i>	Agdash
6231	var. <i>leucurum</i>	Dzhalilabad	6318	var. <i>leucurum</i>	Absheron
6114	var. <i>hordeiforme</i>	Evlakh	6099	var. <i>leucurum</i>	Khanlar
6134	var. <i>alborovinciale</i>	Masalli	6147	var. <i>apulicum</i>	Agsu
6150	var. <i>apulicum</i>	Mingichavur	6232	var. <i>erythromelan</i>	Dzhalilabad
6129	var. <i>melanopus</i>	Nakhchivan	6155	var. <i>apulicum</i>	Nakhchivan
6141	var. <i>leucomelan</i>	Nakhchivan	6159	var. <i>reichenbachii</i>	Zakatala
6108	var. <i>hordeiforme</i>	Barda	6094	var. <i>leucurum</i>	Agsu
6088	var. <i>leucurum</i>	Evlakh	6117	var. <i>hordeiforme</i>	Nakhchivan
6308	var. <i>leucomelan</i>	Absheron	6115	var. <i>hordeiforme</i>	Nakhchivan
6118	var. <i>hordeiforme</i>	Nakhchivan	6112	var. <i>hordeiforme</i>	Shemkir
6142	var. <i>leucomelan</i>	Masalli	6096	var. <i>leucurum</i>	Agsu

Table 1
Continued

1	2	3	4	5	6
6153	var. <i>apulicum</i>	Nakhchivan	6145	var. <i>leucomelan</i>	Nakhchivan
6131	var. <i>murciense</i>	Shamakhi	6095	var. <i>leucurum</i>	Agsu
6093	var. <i>leucurum</i>	Geokchay	6146	var. <i>apulicum</i>	Barda
6116	var. <i>hordeiforme</i>	Nakhchivan	6163	var. <i>erythromelan</i>	Shamakhi
6110	var. <i>hordeiforme</i>	Mingichavur	6316	var. <i>caerulescens</i>	Absheron
6103	var. <i>hordeiforme</i>	Shaki	6122	var. <i>boeufii</i>	Masalli
6091	var. <i>leucurum</i>	Khachmaz	6120	var. <i>hordeiforme</i>	Nakhchivan
6127	var. <i>melanopus</i>	Barda	6143	var. <i>leucomelan</i>	Nakhchivan
6100	var. <i>leucurum</i>	Barda	6104	var. <i>hordeiforme</i>	Tartar
6105	var. <i>hordeiforme</i>	Tartar	6307	var. <i>hordeiforme</i>	Absheron
6315	var. <i>leucurum</i>	Absheron	6102	var. <i>leucurum</i>	Shamakhi
6166	var. <i>niloticum</i>	Dzhalilabad	6090	var. <i>leucurum</i>	Tartar
6312	var. <i>leucurum</i>	Absheron	6113	var. <i>hordeiforme</i>	Gakh
6305	var. <i>leucurum</i>	Absheron	6164	var. <i>affine</i>	Shamakhi
6161	var. <i>erythromelan</i>	Shamakhi	6137	var. <i>leucomelan</i>	Saatli
6126	var. <i>melanopus</i>	Tartar	6317	var. <i>leucurum</i>	Absheron
6111	var. <i>hordeiforme</i>	Akstafa	6306	var. <i>leucurum</i>	Absheron
6311	var. <i>leucomelan</i>	Absheron	6092	var. <i>leucurum</i>	Agdam
6140	var. <i>leucomelan</i>	Kazakh	6097	var. <i>leucurum</i>	Agsu
6106	var. <i>hordeiforme</i>	Tartar	6319	var. <i>alborovinciae</i>	Absheron
6162	var. <i>erythromelan</i>	Shamakhi	6132	var. <i>murciense</i>	Ismailli
6313	var. <i>murciense</i>	Absheron	6107	var. <i>hordeiforme</i>	Shamakhi
6139	var. <i>leucomelan</i>	Bilesuvar	6085	var. <i>leucurum</i>	Akstafa
6125	var. <i>melanopus</i>	Mingichavur	6151	var. <i>apulicum</i>	Nakhichevan
6165	var. <i>niloticum</i>	Agsu	6149	var. <i>apulicum</i>	Khanlar

Table 2
GeneBank number, botanical varieties and geographical origin of bread wheat accessions used in the study

GeneBank number	Sample name	Geographical origination	GeneBank number	Sample name	Geographical origination
1	2	3	4	5	6
7046	var. <i>albidum</i>	Gobustan	6928	var. <i>graecum</i>	Gazakh
6168	var. <i>albidum</i>	Tartar	7045	var. <i>graecum</i>	Gobustan
7041	var. <i>albidum</i>	Gobustan	7049	var. <i>lutescens</i>	Gobustan
7323	var. <i>barbarossa</i>	Nakhchivan	6278	var. <i>lutescens</i>	Absheron
7321	var. <i>barbarossa</i>	Absheron	6300	var. <i>lutescens</i>	Absheron
6286	var. <i>erythroleucon</i>	Absheron	7044	var. <i>lutescens</i>	Gobustan
6285	var. <i>erythroleucon</i>	Absheron	6167	var. <i>lutescens</i>	Tartar
6274	var. <i>erythroleucon</i>	Absheron	6169	var. <i>lutescens</i>	Tartar
6284	var. <i>erythroleucon</i>	Absheron	6291	var. <i>lutescens</i>	Absheron
7051	var. <i>erythrospermum</i>	Gobustan	7050	var. <i>lutescens</i>	Gobustan
7255	var. <i>erythrospermum</i>	Goranboy	6264	var. <i>lutescens</i>	Shaki
9530	var. <i>erythrospermum</i>	Shaki	6293	var. <i>lutescens</i>	Absheron
7254	var. <i>erythrospermum</i>	Shamakhi	6280	var. <i>lutescens</i>	Absheron
9527	var. <i>erythrospermum</i>	Absheron	6347	var. <i>lutescens</i>	Tartar
9526	var. <i>erythrospermum</i>	Garayazy	6277	var. <i>lutescens</i>	Absheron

Table 2
Continued

1	2	3	4	5	6
9528	var. <i>erythrospERMum</i>	Berde	6303	var. <i>lutescens</i>	Absheron
6298	var. <i>erythrospERMum</i>	Absheron	6288	var. <i>lutescens</i>	Absheron
9525	var. <i>erythrospERMum</i>	Garayazy	6283	var. <i>lutescens</i>	Absheron
9529	var. <i>erythrospERMum</i>	Garayazy	6302	var. <i>lutescens</i>	Absheron
6296	var. <i>erythrospERMum</i>	Absheron	6350	var. <i>lutescens</i>	Tartar
9533	var. <i>erythrospERMum</i>	Oghuz	6301	var. <i>lutescens</i>	Absheron
7256	var. <i>erythrospERMum</i>	Shaki	6275	var. <i>lutescens</i>	Absheron
6265	var. <i>erythrospERMum</i>	Shaki	7043	var. <i>lutescens</i>	Gobustan
6268	var. <i>erythrospERMum</i>	Shaki	7052	var. <i>lutescens</i>	Gobustan
9531	var. <i>erythrospERMum</i>	Shaki	6292	var. <i>lutescens</i>	Absheron
6299	var. <i>erythrospERMum</i>	Absheron	6266	var. <i>lutescens</i>	Shaki
6297	var. <i>erythrospERMum</i>	Absheron	7042	var. <i>lutescens</i>	Gobustan
6290	var. <i>erythrospERMum</i>	Absheron	6170	var. <i>lutescens</i>	Tartar
9532	var. <i>erythrospERMum</i>	Oghuz	6263	var. <i>lutescens</i>	Shaki
6287	var. <i>ferrugineum</i>	Absheron	9542	var. <i>lutescens</i>	Gobustan
6289	var. <i>ferrugineum</i>	Absheron	7319	var. <i>meridionale</i>	Nakhchivan
6279	var. <i>ferrugineum</i>	Absheron	7320	var. <i>meridionale</i>	Lerik
7245	var. <i>graecum</i>	Nakhchivan	6276	var. <i>meridionale</i>	Absheron
6927	var. <i>graecum</i>	Masally	6932	var. <i>milturum</i>	Shamakhi
6413	var. <i>graecum</i>	Gobustan	7249	var. <i>milturum</i>	Absheron
7246	var. <i>graecum</i>	Nakhchivan	6931	var. <i>milturum</i>	Absheron
6920	var. <i>graecum</i>	Samukh	7250	var. <i>milturum</i>	Baku
6349	var. <i>graecum</i>	Tartar	6930	var. <i>milturum</i>	Khankandi
6295	var. <i>graecum</i>	Absheron	7253	var. <i>milturum</i>	Nakhchivan
7038	var. <i>graecum</i>	Gobustan	7248	var. <i>milturum</i>	Devechy
6351	var. <i>graecum</i>	Tartar	7247	var. <i>milturum</i>	Masalli
6262	var. <i>graecum</i>	Shaki	7251	var. <i>milturum</i>	Baku
7040	var. <i>graecum</i>	Gobustan	7252	var. <i>milturum</i>	Baku
6929	var. <i>graecum</i>	Lerik	7039	var. <i>outessa</i>	Gobustan
9521	var. <i>graecum</i>	Oghuz	8738	var. <i>velutinum</i>	Absheron
6926	var. <i>graecum</i>	Kurdemir	6282	var. <i>velutinum</i>	Absheron
7244	var. <i>graecum</i>	Nakhchivan	6281	var. <i>velutinum</i>	Absheron
6348	var. <i>graecum</i>	Tartar	6408	var. <i>velutinum</i>	Gobustan
6294	var. <i>graecum</i>	Absheron	7047	var. <i>velutinum</i>	Gobustan

Molecular analysis

DNA extraction was performed according to the CTAB (cetyltrimethylammonium bromide) protocol proposed by Doyle and Doyle (1987) with some modifications. The concentration and purity of the DNA molecule were determined by NanoDrop (Thermo, NANO DROP, 2000). DNA amplification was performed in a reaction volume of 20 µl, containing 2 µl 10x PCR buffer, 2 µl mixture dNTP (5 mM), 1.5 µl MgCl₂ (50mM), 2 µl of each primer (15 pmol/µl), 0.1 µl of Taq-polymerase enzyme (1 U/µl) and 2 µl of extracted DNA

(50 ng/µl). As a result of the optimization following amplification conditions were selected: pre-denaturation at 94°C for 5 minutes; 35 cycles of – denaturation at 94°C (1 min), annealing for 45 seconds (temperature depended on the primer used), elongation for 5 minutes at 72°C; the final elongation at 72° C for 10 minutes. Amplification was performed in a thermal cycler T100 (AppliedBiosystems, USA). Electrophoresis of PCR products was performed on 2% agarose gel by adding ethidium bromide and visualized under UV light using gel documentation system BioRad.

Statistical data analysis

Analysis of amplified fragments was performed using the computer program PAST (Hommer et al., 2001). To identify the genetic diversity among the studied genotypes got data were presented in a binary matrix form where “the presence” or “absence” of certain PCR fragments were noted as 1 and 0, respectively. The genetic diversity index was calculated according to the formula of Weir (1990):

$$H = 1 - \sum_i^n p_i^2$$

where H – index of genetic diversity, p_i – frequency of alleles. Construction of the dendrogram and evaluation of genetic similarity between the sample pairs was performed according to Nei’s genetic distance index, using PAST program.

Results and Discussion

Twenty ISSR primers were preliminary tested for molecular-genetic analysis of hard and soft wheat accessions, of which the 8 most informative markers that produce clear and strictly reproducible results were selected for further experiments (Table 3). The main area of distribution of fragments was located in the range of 100-2000 bp. A total of 107 and 136 PCR fragments were identified for durum and bread wheat genotypes, respectively. Primers UBC864 and UBC841 revealed the highest number of amplified bands in bread wheat accessions. In durum wheat accessions the maximum value was noted for UBC841 and UBC857. The number of fragments amplified by these primers was 17 and 18 respectively. In average ISSR primers produced 17 bands for bread and 13.3 bands for durum wheat genotypes. Gel electrophoresis of PCR profiles using ISSR primers showed

that some genotypes of both soft and hard wheats, possess unique fragments of different length. For example, among durum wheat samples, using primer UBC 841 the unique fragment of 110 bp was noted for Barakatli and var. *leucomelan* (6145). ISSR-fragment of 700 bp amplified with primer UBC112 was presented only in genotypes var. *hordeiforme* (6305) and Mirbeshir 50. Also, it should be noted that a fragment of 980 bp synthesized by primer UBC 873 was found only in genotype var. *leucomelan* (6145).

Used ISSR primers also enabled to obtain unique amplicons for soft wheat genotypes and were highly informative. In particular, using primer UBC 841 specific band of 850 bp was only synthesized in genotype var. *millitutum* (7248). For accession belonged to var. *erythrosperrum* (6290) was presented a unique fragment of 2200 bp (with primer UBC857). Also, the primer UBC864 identified fragments of 2000 bp specific for var. *erythrosperrum* (6290), 150 bp for var. *meridionale* (6276) and 1800 bp for var. *albidum* (7041). These fragments can be used for identification of each above-mentioned sample. Thus, these bread and durum wheat samples were selected as unique genotypes.

According to our observations, high polymorphism was revealed for the majority of the amplified fragments. The percentage of polymorphic fragments among durum wheat genotypes ranged from 63.6 to 88.9% and averaged 82%, which was quite high. Polymorphism revealed in this study is in agreement with other wheat studies. Sotalian et al. (2008) in their studies of 39 local wheat varieties of Iranian origin with 15 ISSR markers also found higher polymorphism (82.2%). High polymorphism observed in our experiments can be explained by the fact that microsatellites are characterized by a high rate of sequence changes due to point mutations and “slippage” during DNA replication. The opposite of the above noted results, Carvalho et al. (2008) in the study of 51

Table 3
ISSR primers and their statistical parameters

Primers	Sequence (5’-3’)	Number of total fragments		Number of polymorphic fragments		Percentage of polymorphism		Genetic diversity index	
		<i>T. aestivum</i>	<i>T. durum</i>	<i>T. aestivum</i>	<i>T. durum</i>	<i>T. aestivum</i>	<i>T. durum</i>	<i>T. aestivum</i>	<i>T. durum</i>
UBC112	(GACA) ₄	13	9	10	8	77	88.9	0.92	0.96
UBC808	(AG) ₈ C	18	13	13	11	72	84.6	0.94	0.82
UBC811	(GA) ₈ C	19	12	10	10	53	83.3	0.69	0.97
UBC827	(AC) ₇ G	15	16	15	12	100	75.0	0.83	0.96
UBC841	G(ACA) ₄ (GC) ₃ C	20	17	13	14	65	82.4	0.96	0.96
UBC857	(AC) ₈ TT	19	18	14	16	74	88.9	0.98	0.98
UBC864	TAG(GT) ₆ GAA	22	11	13	7	59	63.6	0.98	0.88
UBC873	(GACA) ₄	10	11	9	9	90	81.8	0.96	0.97
Total		136	107	97	87				
Average		17	13.3	17	10.9	74	82.0	0.91	0.94

durum wheat varieties of belonging to 26 different botanical species, revealed a low level of polymorphism (42.1%).

Song et al. (2002) in the study of microsatellite markers have found that the motifs with three or four nucleotides show the higher level of polymorphism. Furthermore, Nagoaka and Ogihara (1997) observed maximum polymorphism for primers with tetra nucleotide repeats in hexaploid wheat collection. In our studies primer UBC811 containing (GA)_n repeats, showed relatively low level of polymorphism (83.3%) in durum and in bread wheats (53%). For primer UBC 827 and UBC 857 having in its structure dinucleotide repeat (AC)_n, were identified middle and high level of polymorphism for both soft (74% and 100%) and durum wheat (75% and 88.9%), respectively. Among the set of primers, two primers UBC112 and UBC873 with 4-nucleotide repeats (GACA)_n showed the highest level of polymorphism for bread (90% and 77%) and durum (88.9% and 81.8%) wheats. One primer UBC864, comprising (GT)_n repeats, found a relatively low level of polymorphism among hard (63.6%) and soft wheat (59%) genotypes.

In this study genetic diversity index (GD) was calculated for each ISSR locus. The level of genetic diversity for durum wheat samples ranged from 0.82 to 0.98 and averaged 0.94. The high value of GDI can be explained by the involvement in the study of material from different geographical regions of Azerbaijan. Primers UBC857, UBC811 and UBC873, with respect to all studied primers had the highest rate of genetic diversity (Table 3). It is important to highlight the primer UBC857, which, along with the highest GDI (0.98) showed a high level of polymorphism (88.9%). Also, according to the PCR results the primer was distinguished with the highest number of amplified bands. The average value of the genetic diversity index for bread wheat was 0.91 and ranged from 0.68 to 0.98. Similar to the durum wheat, primers UBC857 (0.98), UBC 873 (0.96) and UBC864 (0.98) were differed for higher value of genetic diversity index in bread wheat as well. As noted above, among the studied collection of durum wheat by the number genotypes predominated botanical varieties were var. *leucomelan*, var. *leucurum*, var. *hordeiforme* and var. *apulicum*, in bread wheat var. *erythro-leucon*, var. *erythrosperrum*, var. *graecum*, var. *lutescens*, var. *milturum* and var. *velutinum*. In durum wheat samples the highest rate of GDI (0.88) was detected among the 19 genotypes of var. *hordeiforme*. Despite the relatively small number of genotypes belonged to var. *melanopus*, GR index was high for these varieties (0.83), indicating the rich genetic diversity of current breeding material. Samples of var. *melanopus* were cultivated in certain areas (collection cites), differing in the soil and climatic characteristics that may contribute to their genetic differentiation. In bread wheat a

high index of genetic diversity has been identified among 20 genotypes of var. *erythrosperrum* (0.90). In spite of the small number of genotypes of var. *velutinum* the genetic diversity index was 0.77. The highest number of genotypes (28) was for var. *lutescens* with GRI equal to 0.89.

The results are in good agreement with previously published data on ISSR analysis of wheat genotypes. For example, Pasqualone et al. (2000) tested the effectiveness of ISSR markers to identify the differences between the 30 local Italian durum wheat and 22 breeding lines and found that the two primers is sufficient to detect differences between all the samples studied.

In the present study cluster analysis was conducted and a tree showing genetic relationships between samples was constructed. Results of cluster analysis of hard and soft wheat enabled to allocate 11 and 12 main clusters, respectively and revealed the complex nature of the distribution of the studied varieties and botanical varieties (Figure 1, 2). Each cluster is grouped genotypes from different regions of the country and of different botanical varieties. As a result of grouping of durum wheat genotypes cluster 7 proved the most numerous, where 36.6% of all studied samples were localized. Of the 40 genotypes formed the eighth cluster mainly dominated var. *leucurum*, var. *hordeiforme* and var. *apulicum*. Furthermore, five independent clusters were identified in the dendrogram. Genotypes var. *apulicum* of Khanlar regions (6149) and Aghsu (6147) formed a cluster 10 and 11, respectively, var. *alborovinciale* (6319) of the Absheron region a cluster 9, var. *leucurum* (6094) from Aghsu a cluster 1 and var. *murciense* (6131) from the Shamakhy region concentrated in a cluster 6.

This indicates the uniqueness of these genomes compared to a set of genotypes studied. The most remote was var. *apulicum* of Aghsu region (6147), which is completely separated from all other samples and was located in a separate cluster. The least numerous cluster 4 is presented by only two genotypes: var. *erythromelan* (6161) from Shamakhy and var. *melanopus* (6126) from Tartar region. It should be noted that these areas have similar soil and climatic conditions, what in our view, may contribute to their similar genetic organization. Some tendency to unite species originating from the same region was observed. For example, the genotypes of var. *leucurum* (6317 and 6306) of Absheron were distributed in the cluster 8, which is quite logical. The same pattern was observed for samples var. *hordeiforme* (6117 and 6115) from the Nakhichevan region. In addition to the botanical varieties each cluster incorporates various varieties of durum wheat. Five of 10 varieties of durum wheat (Garagylychyg, Agh bugda, Sharg, Shirvan and Tartar) were grouped in cluster 7. The remaining varieties were scattered in various clusters,

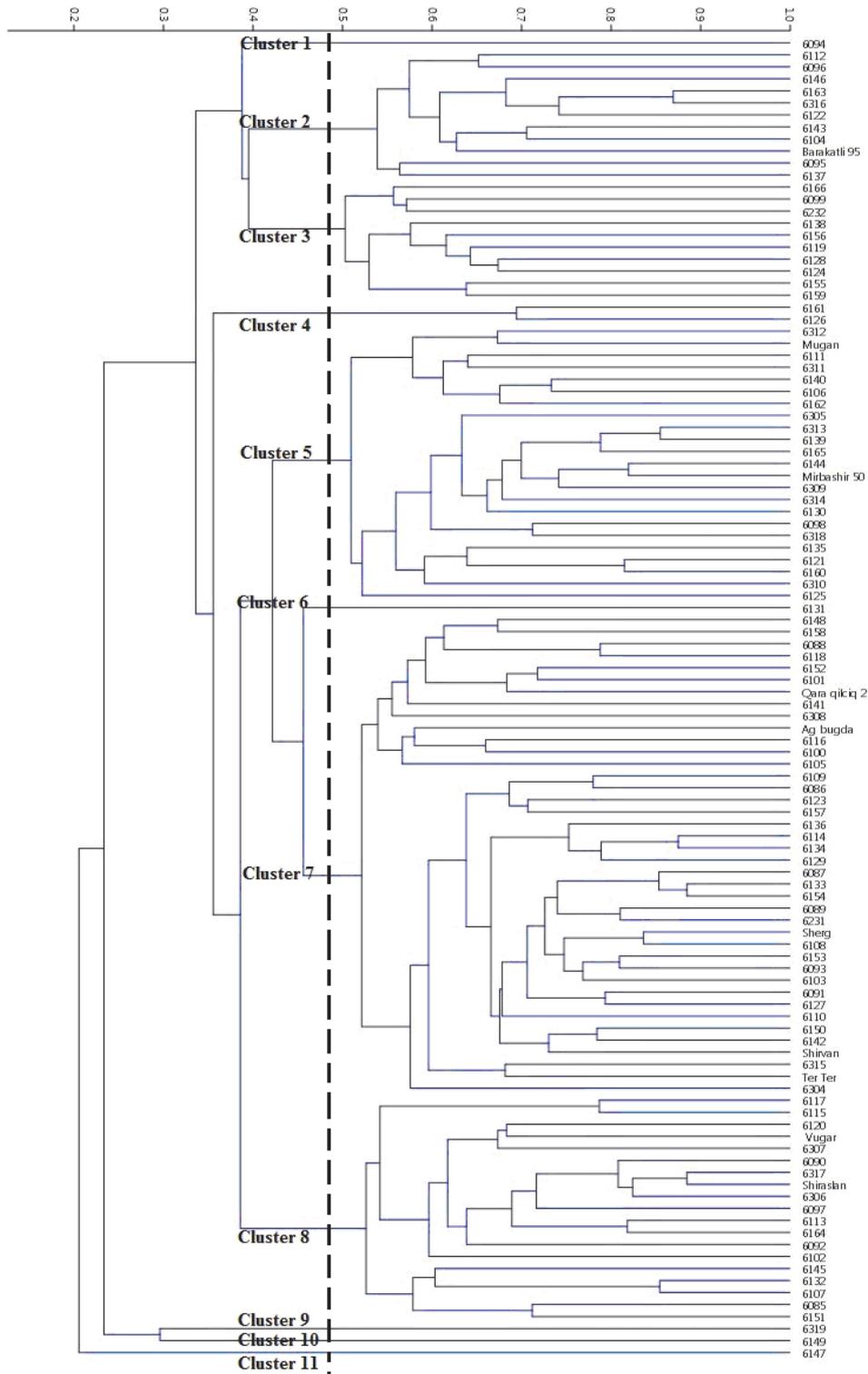


Fig. 1. Dendrogram of durum wheat genotypes based on ISSR analysis

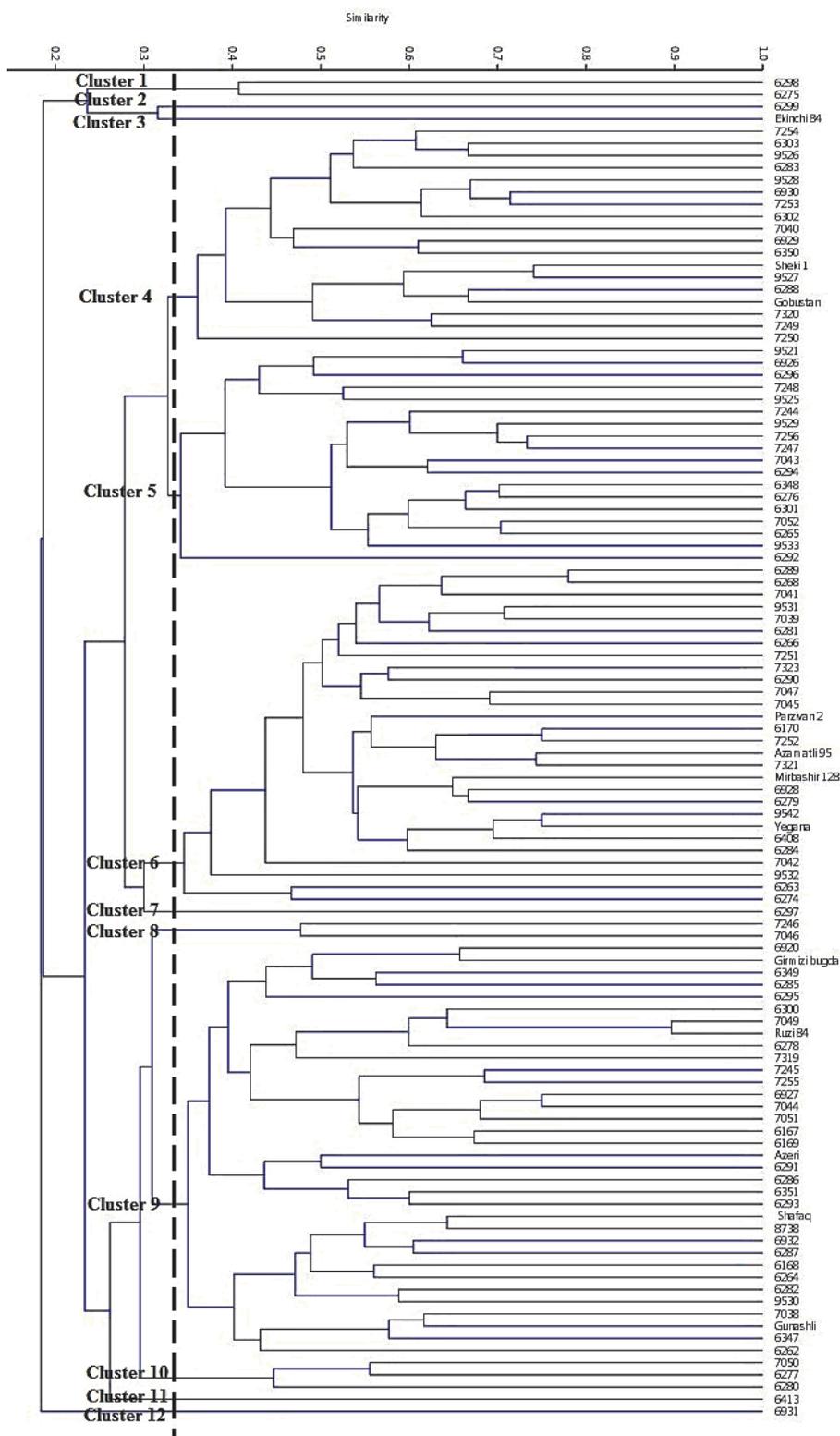


Fig. 2. Dendrogram of bread wheat genotypes based on ISSR analysis

which is probably explained by the peculiarities of selection, as ISSR loci are not a subject for breeding.

As a result of the cluster analysis of soft wheat 5 samples formed a separate cluster as divergent forms. The most numerous cluster 9, which contained 30.9% of the studied genotypes. Among the 34 genotypes mostly prevalent botanical varieties were var. *lutescens* (10) and var. *graecum* (7). In this group, the most genetically close accessions appeared to be samples of var. *graecum* (6927) of the Gobustan region and var. *lutescens* (6167) of the Tartar region, for which the value of genetic distance index was equal to 0.12. Genotypes var. *albidum* (Tartar) and var. *graecum* (Absheron) have been identified as the most distant accessions, with genetic distance index equal to 0.90. Cluster 7 combines 4 accessions of var. *erythrosperrum* and 4 var. *lutescens*. In this cluster is the most genetically similar proved to be genotypes of var. *erythrosperrum* (6290) and var. *lutescens* (6260) (GDI=0.18), the most remote – var. *erythrosperrum* (7255) and (var. *ferrugineum*) (43) with GDI equal to 0.78. Cluster 6, comprising 25.5% of genotypes, contained both botanical varieties and realized varieties (Azamatly 95, Parzivan 2, Mirbashir 128, Yegana). In 4 and 5 clusters were localized 18 genotypes, in the 5 mainly distributed genotypes of var. *erythrosperrum*, in the 4 – var. *milturum*. It should be noted that among the 110 studied genotypes of bread wheat, Ekinchi 84, accessions belonged to var. *erythrosperrum* (Absheron), var. *milturum* (Absheron), var. *graecum* (Gobustan) and var. *milturum* (Baku), separated from the rest of the genotypes and formed separate clusters. These genotypes were estimated as the most genetically divergent.

Thus, on the basis of the above mentioned, we can give the following description of ISSR dendrogram:

1. The complex nature of the distribution of genotypes of soft and durum wheat;
2. The absence of a particular relation between genetic structure of genotypes and their geographical distribution, that is, the differentiation of botanical varieties and varieties of hard and soft wheat had no clear geographical focus;
3. The presence of high genetic diversity among all studied collection and within the clusters formed;
4. Identification of clusters with unique genotypes of durum wheat.

Identification of the genetic relationship among different genotypes of wheat was noted in many research papers. For example, Carvalho et al. (2008) in the study of 48 varieties of bread wheat from the Old Portuguese collection using 18 ISSR markers found that the majority of crops belonging to the same botanical species were grouped in the same cluster. These results are also in consistent with the work of Malik et al (2010), who studied 27 genotypes of bread wheat. As a

result of the cluster analysis, genotypes grouped according to their origin.

Summarizing all the above, it was suggested that ISSR markers could be successfully used to detect the DNA polymorphism in durum and bread wheat collections. The most remote genotypes identified as a result of cluster analysis may be recommended for use by breeders to produce genetically diverse populations with a maximum range of variation in the hybrid offspring.

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