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# Genotyping by Sequencing and Rust Resistance of Azerbaijani Durum Wheat Germplasm

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**Abstract**

Genotyping-by-sequencing (GBS) is a genetic screening method for discovering and genotyping novel single nucleotide polymorphisms (SNPs) in crop genomes and populations. In the current research a phenotypic and genotypic assessment of 76 durum wheat (Triticum durum Desf.) accessions of Azerbaijan origin was made using six phenotypic traits and GBS technology. After screening for leaf and stem rust resistance at the seedling stage, 16 genotypes displayed resistance to leaf rust and 14 to stem rust. Some relationships were found between resistance to leaf rust and phenotypic traits of botanical varieties. The highest Pearson's correlation ($r=0.53$; $p < 0.001$) was noted between awn color and pubescence. The durum wheat genotypes fell into four main groups in the clustered heat map; grouping according to botanical variety. A total of 748 SNP markers were obtained for the collection. The average polymorphic information content and genetic diversity index for the entire collection were 0.329 and 0.420, respectively. With respect to population structure, two and three subpopulations were identified. The principal component and cluster analyses resulted very comparable to the population structure analysis at $k = 3$. Clustering analysis based on GBS data showed the genotypes divided into six clusters. Some consistency was noted between the grouping of genotypes and their pedigrees, and the botanical variety. The results could facilitate durum wheat collection, conservation, breeding and will open the door for future association mapping studies. In addition, the resistant genotypes can be utilized as donors to broaden the genetic base of rust resistance in wheat breeding.

**Keywords:** Phenotypic; Genetic; Breeding

**Abbreviations:** ANAS, Azerbaijan National Academy of Sciences; CTAB, Cetyl trimethylammonium bromide; GBS, Genotyping-by-sequencing; GWAS, genome-wide association studies; MAF, minor allele frequency; NGS, Next Generation Sequencing; PCA, principal component analysis; RFLP, restriction fragment length polymorphisms; SNP, Single nucleotide polymorphism; UNEAK, Universal Network Enabled Analysis Kit;

## Introduction

Wheat is represented by a wide diversity of species, among which the most economically important species are *Triticum aestivum* L. (bread wheat) and *Triticum durum* Desf. Durum wheat is the most important tetraploid wheat specie (2n = 4x = 28, AABB) with a genome size of approximately 12 Gb. The domestication of durum wheat dates back 8,000 - 12,000 years in southwest Asia [1] through intergeneric hybridization and polyploidization. Today, durum wheat is grown on about 10% of the world's wheat growing area, mostly in western Asia, northern and eastern Africa, the North American Great Plains, India, and eastern and Mediterranean Europe. The peculiar characteristics of this crop, such as large kernel size, hardness, and a bright white to yellow color, make it suitable for the production of a wide range of end products, including pasta, different kinds of leavened and unleavened breads, couscous, and bulgur [2].

Breeding activities have reduced the genetic diversity of cultivated crops. The AABB tetraploid wheats may have lost around 50% of their gene diversity during the transition from *T. turgidum* subsp. *dicoccoides* (Körn. Ex Asch. & Graebn.) Thell. to *T. turgidum* subsp. *dicoccum* Schrank. In addition, a strong selection pressure accompanied the transition from *T. turgidum* subsp. *dicoccum* to *T. turgidum* subsp. *durum* (Desf.) Husn. [3]. Breeding practices further reduced the genetic diversity of modern durum wheat cultivars. Use of the local genepool from the Fertile Crescent and nearby countries has contributed to a higher level of variability for many traits across thousands of generations may have compensated for the low level of diversity of *T. durum* [2].

Azerbaijan is one of the main areas in the Asiatic center of origin of cultivated plants [4] and is characterized by a wide diversity of climates. The country is rich in endemic wild and cultivated species of *Triticum, Aegilops,* and *Secale* and is considered as one of the primary gene centers of speciation of the genus *Triticum* [5]. Archaeological and paleontological studies show that the cultivation of wheat in Azerbaijan began more than 6-8 thousand years ago. The durum wheat of Azerbaijan is rich in morpho-botanical composition. The wheat genepool in Azerbaijan is enriched by numerous spontaneous hybrids, natural mutants, varieties of folk selection, and rare botanical varieties [6]. Many modern varieties were developed using this genepool, as well as introduced material. Now, more attention is paid to the description and conservation of biodiversity that is concentrated mainly in old varieties and populations. Today, over 2,000 accessions are being conserved in the National Genebank created in 2003 at Genetic Resources Institute of Azerbaijan National Academy of Science (ANAS). The local wheat germplasm is adapted to a wide range of environments and carries a large reservoir of useful genes, such as resistance to the abiotic and biotic factors, including heat and drought tolerance, and stem, yellow, and leaf rust resistance, all major constraints to wheat production. The study of this heritage makes it possible to determine the genetic diversity and identify genotypes that are useful donors of rare alleles of genes of economically significant traits.

Several types of molecular markers, ranging from restriction fragment length polymorphisms (RFLPs) to high-density single nucleotide polymorphisms, have been identified and effectively utilized in wheat for genetic diversity analysis, complex trait dissection, and marker-assisted breeding [7,8]. The advent of next-generation

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sequencing (NGS) technologies has led to remarkable advances in the field of genomics, enabling fast and cost-effective generation of ultra-throughput sequence data with exquisite resolution and accuracy. Genotyping-by-sequencing can further broaden NGS use in large crop genomes such as wheat [9]. GBS is a genetic screening method for discovering and genotyping novel SNPs in crop genomes and populations. The method reduces genome complexity with restriction enzymes and a subset of restriction fragments is sequenced to produce partial, but genome-wide, sequencing reads [10]. The GBS data can be easily implemented in genome-wide association studies (GWAS), genetic linkage analyses, genomic diversity studies, and genomic selection. No prior knowledge of the species genomes is required [11] making this method accessible for genomic studies of various species. GBS has been applied to multiple crops including wheat [12,13], barley [14] maize [15] chickpea [16] lentil [17] and cotton [18].

The objectives of the study were to evaluate the relationship among some morphological traits and resistance to leaf and stripe rust and to assess the genetic diversity in a local durum wheat collection of Azerbaijan using GBS technology.

## Materials and Methods

### Plant Materials

Seventy-six durum wheat accessions, including 16 varieties, belonging to 13 botanical varieties from Azerbaijan, were used. One introduced variety (Langdon) was also included. The accessions were obtained from the National Genebank under the Genetic Resources Institute of ANAS. Accession numbers, collection sites and botanical varieties of the studied genotypes are given in Table 1.

### Phenotype Screening

Seedlings were grown in 20 × 20 × 5 cm aluminum pans in the greenhouse at 20 ± 4 °C and ambient light. Rust spores were thawed and heat-shocked for 6 min in a water bath at 42 °C just prior to use. Spores were suspended in Soltrol 170 isoparaffin oil at a concentration of approximately 5 × 106/ml and sprayed onto seedlings at the two-leaf stage. The oil was allowed to evaporate for at least 10 min, and then the plants were placed in a dew chamber at 20 ± 1 °C with 100% relative humidity in the dark for approximately 16 hours. For stem rust, the dew chamber was illuminated for the last hour to stimulate

**Table 1:** The list of genotypes included in the study.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | 6086 | var. *leucurum* | Gazakh | 39 | 6138 | var. *leucomelan* | Yevlakh |
| 2 | 6087 | var. *leucurum* | Aghdara | 40 | 6139 | var. *leucomelan* | Belasuvar |
| 3 | 6088 | var. *leucurum* | Yevlakh | 41 | 6140 | var. *leucomelan* | Gazakh |
| 4 | 6090 | var. *leucurum* | Terter | 42 | 6141 | var. *leucomelan* | Nakhchivan |
| 5 | 6091 | var. *leucurum* | Khachmaz | 43 | 6142 | var. *leucomelan* | Masalli |
| 6 | 6092 | var. *leucurum* | Aghdam | 44 | 6144 | var. *leucomelan* | Lerik |
| 7 | 6093 | var. *leucurum* | Goychay | 45 | 6146 | var. *apulicum* | Barda |
| 8 | 6094 | var. *leucurum* | Aghsu | 46 | 6147 | var. *apulicum* | Aghsu |
| 9 | 6098 | var. *leucurum* | Aghdash | 47 | 6148 | var. *apulicum* | Ismayilly |
| 10 | 6099 | var. *leucurum* | Khanlar | 48 | 6149 | var. *apulicum* | Khanlar |
| 11 | 6100 | var. *leucurum* | Barda | 49 | 6150 | var. *apulicum* | Mingechevir |
| 12 | 6101 | var. *leucurum* | Tovuz | 50 | 6151 | var. *apulicum* | Nakhchivan |
| 13 | 6102 | var. *leucurum* | Shamakhi | 51 | 6159 | var. *areichenbachii* | |
| 14 | 6103 | var. *hordeiforme* | Shaki | 52 | 6160 | var. *areichenbachii* | |
| 15 | 6104 | var. *hordeiforme* | Terter | 53 | 6161 | var. *erythromelan* | Shamakhi |
| 16 | 6107 | var. *hordeiforme* | Shamakhi | 54 | 6163 | var. *erythromelan* | |
| 17 | 6108 | var. *hordeiforme* | Barda | 55 | 6164 | var. *affine* | |
| 18 | 6109 | var. *hordeiforme* | Aghdam | 56 | 8002 | var. *erythromelan* | Shamakhi |
| 19 | 6111 | var. *hordeiforme* | Akstafa | 57 | 8004 | var. *caerulescens* | Absheron |
| 20 | 6112 | var. *hordeiforme* | Shamkir | 58 | 8005 | var. *caerulescens* | vill. Bayan |
| 21 | 6113 | var. *hordeiforme* | Gakh | 59 | 8007 | var. *obscurum* | Absheron |
| 22 | 6114 | var. *hordeiforme* | Yevlakh | 60 | 8008 | var. *obscurum* | Absheron |
| 23 | 6116 | var. *hordeiforme* | Nakhchivan | 61 | Agh bughda | var. *leucurum* | |
| 24 | 6121 | var. *boeufii* | Shamakhi | 62 | Arandeni | var. *apulicum* | |
| 25 | 6122 | var. *boeufii* | Masalli | 63 | Barakatli 95 | var. *hordeiforme* | |
| 26 | 6123 | var. *melanopus* | Tovuz | 64 | Bozak | var. *hordeiforme* | |
| 27 | 6124 | var. *melanopus* | Goranboy | 65 | Jafari | var. *leucurum* | |
| 28 | 6125 | var. *melanopus* | Mingechevir | 66 | Karakilchik | var. *melanopus* | |
| 29 | 6126 | var. *melanopus* | Tartar | 67 | Karakilchiq 2 | var. *apulicum* | |
| 30 | 6129 | var. *melanopus* | Nakhchivan | 68 | Langdon | | USA |
| 31 | 6130 | var. *murciense* | Saatli | 69 | Mirbashir 50 | var. *leucurum* | |
| 32 | 6131 | var. *murciense* | Shamakhi | 70 | Sari bughda | var. *hordeiforme* | |
| 33 | 6132 | var. *murciense* | Ismayilly | 71 | Sharg | var. *leucurum* | |
| 34 | 6133 | var. *alborovinciale* | Jalilabad | 72 | Shiraslan 23 | var. *leucurum* | |
| 35 | 6134 | var. *alborovinciale* | Masalli | 73 | Shirvan bughda | var. *hordeiforme* | |
| 36 | 6135 | var. *leucomelan* | Aghjabadi | 74 | Tartar | var. *provinciale* | |
| 37 | 6136 | var. *leucomelan* | Devechy | 75 | Tartar 2 | var. *provinciale* | |
| 38 | 6137 | var. *leucomelan* | Saatli | 76 | Vugar | var. *leucurum* | |

infection. Plants were moved to a growth chamber at 20 ± 1 °C with a 16-hour photoperiod and a light intensity of 300–400 µmol/m²/sec for symptom development. Infection types (ITs) were recorded using a 0 to 4 scale [19] at 12 days (leaf rust) or 14 days (stem rust) post-inoculation. Plants were inoculated with leaf rust races BBBDB, MFBJG, TTRSD, and MRDSD and stem rust races MCCFC, TPMKC, and RKQQC.

### DNA Extraction and Genotyping-by-Sequencing (GBS)

Young leaves for each accession were collected and lyophilized for DNA extraction, which was carried out using the CTAB procedure described by Doyle[20]. The GBS libraries were constructed in 95-plex and genomic DNA was co-digested with the restriction enzymes *Pst*I (CTGCAG) and *Msp*I (CCGG) and barcoded adapters were ligated to individual samples. Samples were pooled by plate into libraries and polymerase chain reaction amplified. Each 95-plex library was sequenced to 100 bp on a single lane of an Illumina HiSeq 2500. Sequence results were analyzed using the UNEAK GBS pipeline, which is part of the TASSEL 3.0 bioinformatics analysis package.

## Statistical Analysis

SNP markers were filtered from missing data and minor allele frequency (MAF). Samples or SNPs with more than 40% missing data were excluded from the analysis. SNPs with a MAF less than 10% were excluded from further analysis. Correlations between phenotypes and clustering based on the phenotypic data and the principal component analysis (PCA) were calculated and plotted using R software. Nei genetic distances [21] among individuals and botanical varieties were calculated using PoweMarker V3.25 software followed by neighbor-joining clustering [22]. Population structure was estimated using ADMIXTURE software. A hundred replicates were run with a K value between 2 and 10 plus 10 cross-validations [23]. SNP markers were pruned at $r^2$ value equal to 0.5 using PLINK software [24] to avoid bias in ADMIXTUE analysis due to the dependency among SNPs. The smallest K value that had significantly lower cross-validation values in 100 replicates were compared to the next K was considered as the most probable number of ancestral subpopulations in our analysis. Each individual was assigned to the subpopulation that had the highest contribution to its genome. The corset was developed using PowerCore software with default options [25].

## Results and Discussion

### Phenotypic Characterization of *T. durum*

Rust diseases of cereals are potentially serious, most harmful and account for great losses in wheat production. The primary gene centres of wheat also are postulated as the homeland of the most destructive wheat rust pathogens [26,27] noted that the spectra of fungal races and biotypes are more heterogeneous in Transcaucasia, including Azerbaijan, than elsewhere in the Soviet Union. Thus, the most promising sources of defense against diseases can be selected in the home of the host-pathogen system.

In our study, we screened the durum wheat genotypes against both leaf and stem rust. The durum wheat genotypes of Azerbaijan are characterized by a wide range of variability in leaf and stem rust resistance. Phenotypic assessment of 75 durum wheat accessions indicated that 16 were resistant to all tested races of leaf rust, 20 were moderately resistant, and 39 were susceptible. Some relationship was found between resistance to leaf rust and phenotypic traits. A majority of the resistant genotypes belonged to var. *leucurum*, i.e., they had white spikes, awns and seeds. Moreover, 82% of the resistant and 64% of the moderately resistant genotypes had white spikes. On the contrary, of susceptible durum wheat genotypes only 29.7% and 8.1% were characterized with white spike and awn colour, respectively. These genotypes they had red spikes and red or black awns. Therefore, using accessions and/or genotypes of var. *leucurum* with white spikes and awns may be preferred in breeding for leaf rust resistance.

Stem rust, one of the most harmful diseases of wheat, causes the death of a susceptible plant. The wheat genotypes were more susceptible to stem rust compared with leaf rust. [28] evaluated stem rust resistance in 190 Ethiopian bread wheat lines based on phenotypic data from multi-environmental field trials and seedling resistance screening experiments. Eight accessions were resistant to all tested races of stem rust. [29] studied the stem rust resistance level at the seedling stage of the main wheat cultivars in Gansu Province of China. Thirty-eight (50.7%) of the 75 wheat cultivars showed different resistance levels to the six races at the seedling stage. In our collection, only 14 durum wheat accessions were found to be resistant and 12 moderately resistant to stem rust. The remaining genotypes were susceptible.

Four varieties, Shiraslan 23, Vugar, Tartar and Tartar 2, were resistant to both diseases. Tartar and Tartar 2 belong to var. *provenciale* and are recommended for the irrigated plain regions of Azerbaijan, whereas Shiraslan 23 and Vugar, with the same pedigree, can be cultivated in both irrigated and rainfed conditions. These varieties are valuable sources of disease resistance in wheat breeding. In addition, seven genotypes demonstrated resistance to one and moderately resistance to another disease; three genotypes were moderately resistant to both diseases.

A Pearson's correlation analysis showed significant correlations among some pairs of traits (Figure 1). The highest correlation (r=0.53; $p < 0.001$) was noted between awn color and hairness. In addition, as expected, awn color demonstrated a strong positive correlation with spike color (r=0.46) and leaf rust resistance (r=0.38). Spike color also was correlated with leaf rust resistance at a P<0.05* level.

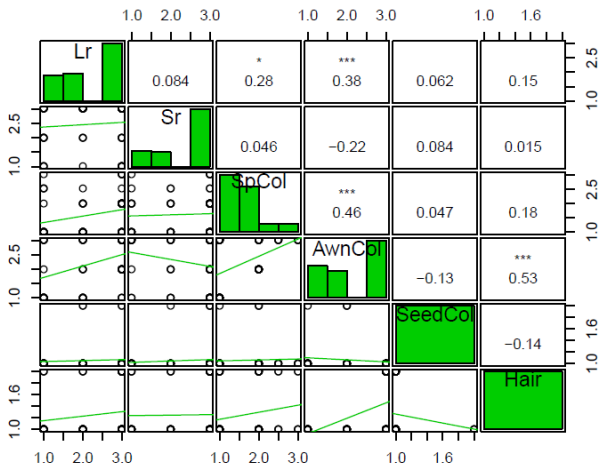


**Figure 1:** A correlation matrix between the studied phenotypic traits. The upper triangle of the matrix shows the Pearson's correlation coefficients, and the lower displays the scatter plots. Lr – leaf rust resistance; Sr – stem rust resistance; SpCol – spike color; AwnCol – awn color; SeedCol – seed color; Hair – hairness; *p < 0.05; *** p < 0.001.

Distinct clusters were noted in the heat map constructed based on the correlation between phenotypic traits and the durum wheat genotypes (Figure 2a). Clustering with six traits formed two major groups (A and B). The first group was further divided into two subgroups with awn colour and hairness in one and leaf rust resistance and spike colour in the other. Stem rust resistance and seed colour were assigned to group B. The 76 durum wheat genotypes fell into four main clusters according to botanical variety. Cluster 1 consisted mainly of var. *leucurum* genotypes and several other varieties all with white spikes, awns and seed colour. Reaction to leaf and stem rust differed among the genotypes. The prevalent reaction to leaf rust was resistant and moderately resistant, whereas a majority of accessions were susceptible to stem rust.

Cluster 2 was comprised of three var. *murciense* and one var. *affine* genotypes, all with red seed colour. Out of five genotypes with red seed colour, four were in cluster 2. The seed colour of the remaining genotypes was white. The reaction to both diseases in cluster 2 was either moderately resistant or susceptible.

Two subclusters were distinguished in cluster 3. The genotypes in subcluster 3A were representatives of four different botanical varieties; all were hairy with black awns and either a white or red spike colour. The genotypes in subcluster 3B were opposite those in subcluster 3A, not hairy and with red awns and spikes; all belonged to var. *hordeiforme*. Most of the genotypes in cluster 3 were susceptible to leaf and stem rust.

Finally, cluster 4 included genotypes from very diverse botanical varieties. The cluster was distinguished by genotypes resistant or moderately resistant to stem rust. The other traits, especially spike colour, were quite variable among genotypes within the cluster. Distribution of leaf rust resistance among the clusters was more inconsistent compared with other traits.

## Population Structure

The principal component analysis (PCA) showed that the first three principal components together explained 24.1% of the total phenotypic variation. For the population structure analysis, the most probable K is either 2 or 3. The cross-validation values over the hundred replicates for K=2 was not significantly different from that for K=3 (two tailed student's t-test), whereas the values for K=3 were significantly lower than those at K=4. However, the PCA analysis was very comparable to the population structure analysis at K=3 (Figure 2b, 2c).

The first subpopulation (green bars in Figure 2c) had the largest number of individuals (46 or 60.5%). Individuals belonging to this subpopulation were the most admixed, with an average contribution of 74.8% for the 46 individuals. All botanical varieties, excluding var. *boeufii* and var. *obscurum*, were included in this subpopulation. The prevailing groups were vars. *hordeiforme* (26%) and *leucurum* (21.7%). Of the 13 var. *hordeiforme* genotypes, 12 were in the first subpopulation.

The second subpopulation (blue bars in Figure 2c) had 24 (31.6%) individuals with an average ancestry contribution of 79.4%. The predominating botanical varieties were *leucurum* (33%) and *lecomelan* (25%); others were represented by one or two genotypes.

The third subpopulation (orange bars in Figure 2c) had the smallest number of individuals (only 6 or 7.9% of the whole germplasm) with

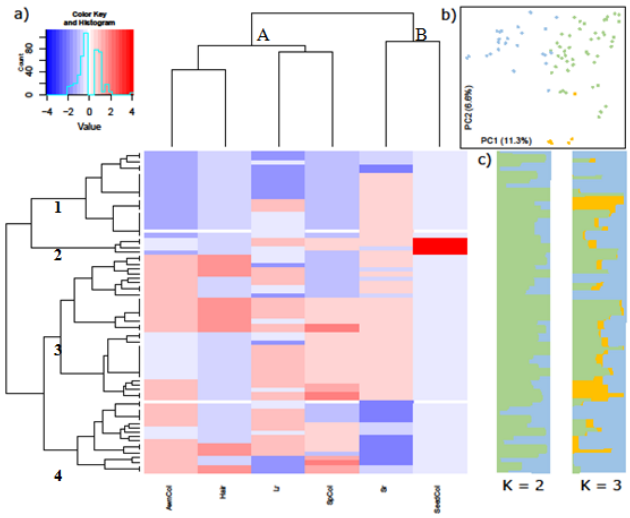


**Figure 2.** a) Clustered heatmap based on phenotypic traits. Each line and column indicates a separate accesion and trait
b) Principal Component Analysis (PCA) for the 76 genotypes; coloring is based on the population structure results
c) Population structure of the germplasm; genotypes were ordered according to the phenotypic clustering.

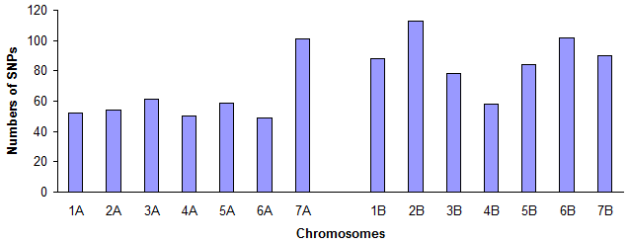


**Figure 3:** Numbers of single nucleotide polymorphisms (SNPs) of durum wheat (*T. durum* L.) chromosomes.

the highest ancestry contribution of 93.5%. Phenotypic clustering seems not to be related to the structure of this germplasm, except that subpopulation 2 (blue) had a greater contribution to phenotypic cluster 4 compared to the other two subpopulations.

## Genetic Diversity and Genetic Relationship

Diversity in plant genetic resources provides opportunity for plant breeders to develop new and improved cultivars with desirable characteristics, which include both farmer-preferred traits (such as yield potential and large seed) and breeders preferred traits (such as pest and disease resistance and photosensitivity) [30].

We obtained a total of 1039 SNP markers for 76 durum wheat genotypes. The genome-wide distribution of SNPs on *T. durum* chromosomes is given in Figure 3. Of the 1039 SNPs, 426 were mapped to A (41%) and 613 to B (59%) genome, the number of SNP markers per chromosomes ranged from 49 to 113. The highest number of markers was identified on 2B (113), while the lowest was on 6A (49) chromosomes. The number of markers per chromosome within the tetraploid genome was 49-101 for the A genome and 58-113 for the B genome, with B/A ratio of 1.4. The results were similar to

previous reports of [31] who indicated no major differences between the A and B genomes.

Majority of SNPs (69.2%) were transition-type, with a transition/transversion SNP ratio of 2.25. Ts/Tv ratio was higher in B genome (2.44) than in the A genome (2.00). A/G and C/T transitions predominated over G/A and T/C transitions. Transition abundance in many species can be explained by the fact that they do not cause the severe distortions of the double helix, and are thus "escape" the repair systems, while transversions are easily recognized by the various repair systems (http://sites.fas.harvard.edu/~bs50/problems10-31key). On the other hand transition mutations more often create synonymous substitutions and are thus less likely to be removed by natural selection.

Out of 1039 SNPs 748 SNP markers with less than 40% missing data and with a MAF more than 10% were used for genetic diversity analysis. The polymorphic information content ranged from 0.203 to 0.375, with an average of 0.329. The genetic diversity index for the whole collection varied between 0.230 and 0.50, the mean value was 0.420. These genotypes represent the different botanical varieties with rich diversity of morphological traits. However, more SNP markers may be required to detect this polymorphism. The genome of allopolyploid species such as wheat is known to be more complicated than that of diploids. Among cereals, the genome of wheat is estimated as one of the least polymorphic, with a SNP detection frequency of about 2.3-3.1 times lower than that of barley [32]. The relatively low level of genetic variation in wheat was previously reported using total seed protein [33] and PCR-based markers [34-36] determined modern Chinese wheat varieties had a lower level of genetic diversity and indicated the potential role of wheat germplasm from abroad and the adoption of distant hybridization in order to enhance the genetic diversity of breeding materials.

A neighbor-joining phylogeny analysis grouped the durum wheat varieties and accessions in a dendrogram based on SNP markers (Figure 4). The genetic distance index ranged from a minimum of 0 (6086-6098; 6135-6136; 6142-6141) to a maximum of 0.99 (Vugar and Agh bughda). In recombination breeding, the most remote genotypes are included in crosses to strengthen heterosis, segregation and variability in the next generations [37].

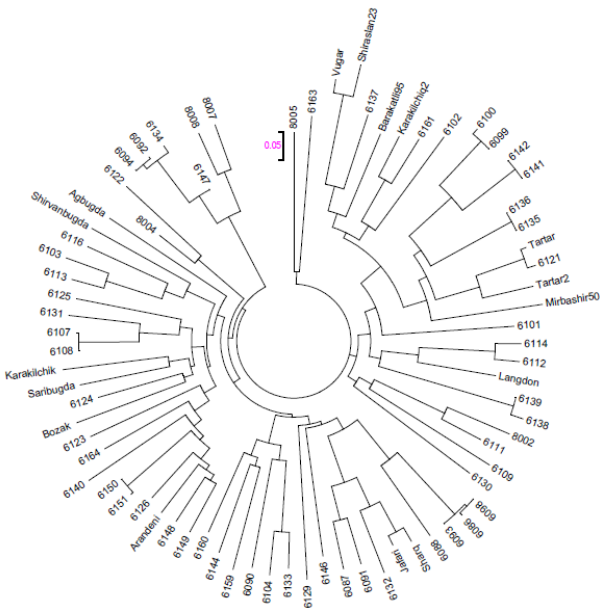


**Figure 4:** Dendrogram of *T. durum* accessions of Azerbaijan using SNP markers.

The dendrogram showed that the genotypes divide into six clusters. Clusters 1 and 5 were the smallest with only two accessions each. Four and six genotypes constituted clusters 3 and 6, respectively. None of the varieties existed in any of these four clusters. Cluster 2 embraces 30.2% of the studied genotypes, eight of which were breeding varieties. Cluster 4 had the maximum number of accessions, with more than half of the studied genotypes (51.3%), including the remaining eight varieties. Of these eight varieties, five were landraces and three breeding varieties.

The grouping pattern observed in the cluster analysis was in agreement with the PCA and STRUCTURE analyses. Clusters 3 and 4 corresponded to the first subpopulation, with minor differences, whereas the same genotypes constituted both cluster 6 and the third subpopulation.

Some relationship was noted between the grouping of genotypes and their botanical classification. Of the nine accessions of var. *lecomelan*, seven were in cluster 2; two pairs (6141 and 6142; 6135 and 6136) were 100% similar and two genotypes (6138 and 6139) exhibited a high degree of genetic relatedness and were clearly distinct from the majority of accessions. In cluster 4, of the 20 var. *leucurum* genotypes, only four were distant from others, and eight formed a nearly homogeneous group together with one var. *murciense* genotype (6132). Among the 13 var. *hordeiforme* genotypes, four formed a uniform group, but the majority placed close to each other in different clusters. Five out of six var. *melanopus* genotypes fell into a separate subgroup of cluster 4, indicating a certain degree of shared alleles. In the same subgroup, a close grouping of five var. *apulicum* accessions was observed. Similar tendencies were observed for two var. *obscurum* and two var. *provensiale* genotypes.

Generally, the clustering results were consistent with the pedigree records. Thus, breeding varieties Vugar and Shiraslan 23 that clustered together were obtained from the same cross between the local variety Sharg and the Mexican variety Oviachik 65. The genetic distance between them was 0.083. Another variety, Barakatli 95 (var. *hordeiforme*), was in the same group with its parent Karakilchig 2 (var. *apulicum*). Tartar 2 was produced from the local variety Tartar through individual selection. These varieties fell into the same tight group with a genetic distance of 0.21. [24] used protein markers to study the genetic relationship among durum wheat landraces and breeding varieties of Azerbaijan. The genotypes Vugar and Shiraslan 23 were quite distant and less related. The varieties Tartar and Tartar 2 were even more dissimilar. These results prove and confirm the superiority of SNP markers over biochemical markers.

However, no linkage was found between the variety Sharg and other varieties (Vugar, Shiraslan 23, and Mirbashir 50) that have Sharg as a parent. On the contrary, Sharg was very closely aligned to Jafary (GD=0.047). Both belonged to var. *leucurum*, but have different pedigrees. In the study of [24], these varieties demonstrated 100% similarity based on gliadin and glutenin markers.

The genetic distance between varieties and all other accessions was 0.057; indicating an expected low differentiation between these two groups. Jafari and Sharg were among the varieties genetically

**Table 2:** Genetic distance indices among botanical varieties.

| Botanical varieties | v. *apulicum* | v. *hordeiforme* | v. *lecomelan* | v. *leucurum* | v. *melanopus* |
|---|---|---|---|---|---|
| v. *hordeiforme* | 0.1985 | | | | |
| v. *lecomelan* | 0.3144 | 0.2526 | | | |
| v. *leucurum* | 0.2054 | 0.1594 | 0.1627 | | |
| v. *melanopus* | 0.1688 | 0.1782 | 0.2840 | 0.2141 | |
| Variety | 0.1747 | 0.1172 | 0.1620 | 0.1113 | 0.1646 |

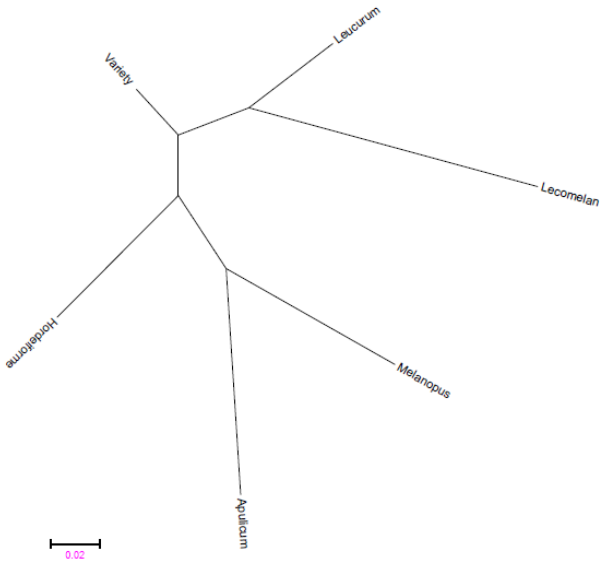


**Figure 5:** Dendrogram showing genetic relationship among botanical varieties of durum wheat.

most similar (GD=0.047). No strong differentiation was noted among studied botanical varieties, however, the distance value was a bit higher. The range was between 0.159 and 0.314 (Table 2). The closest botanical varieties were vars. *leucurum* and *hordeiforme* and vars. *leucurum* and *lecomelan*.

The dendrogram was constructed to visually demonstrate the relationship among botanical varieties (Figure 5). As seen, vars. *leucurum* and *lecomelan* clustered together, with vars. *melanopus* and v*apulicum* forming another branch of the dendrogram. The main difference between vars. *leucurum* and *lecomelan* is awn color, which is white in the first and black in the second. The botanical varieties *melanopus* and *apulicum* are characterized by black awns and white seeds. Both are hairy. They can be distinguished by spike color (white and red, respectively).

In summary, our study revealed a wide range of variation in leaf and stem rust resistance of Azerbaijani durum wheat genotypes. A number of resistant genotypes can be utilized as donors to broaden the genetic base of rust resistance in wheat breeding programs. In addition, a relationship was found between resistance to leaf rust and spike colour, which can accelerate breeding for leaf rust resistance.

The GBS results provide a molecular basis and first SNP data for comprehending genetic diversity in Azerbaijani durum wheat germplasm and will facilitate their conservation strategy. The enrichment of this genetic information with more SNPs will assist future association mapping studies of many traits, including those of the botanical varieties.

## Supplemental Material

A list of genotypes used in the study and additional information on the collection are given in Supplemental Table S1.

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### Conflict Of Interest

The authors declare that they have no conflict of interest.

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