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Genetic Diversity in Durum Wheat Collections of Azerbaijan Based on SSR Markers

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Genetic diversity and relationships among durum wheat (*T. durum* Desf.) accessions and varieties belonged to 29 botanical varieties was studied using simple sequence repeat (SSR) marker system. A total of 104 alleles were produced using 13 SSR primers for 145 durum wheat accessions, with an average of 8 bands per primer. The mean H_E and PIC values were 0.62 and 0.58, respectively, which indicates high diversity in the studied collection. The higher diversities were obtained for var. *leucurum* (PIC=0.57) and var. *hordeiforme* (PIC=0.51). No clear grouping pattern was revealed based on botanical varieties, with few exceptions, indicating a significant amount of shared alleles among them. The highest similarity was noted between var. *hordeiforme* and var. *melonopus*, while var. *leucurum* and var. *melanopus turgidoid* were the most distant. Rich diversity revealed among durum wheat cultivars and botanical varieties can be used as a valuable source for future breeding programs.

Keywords: *Durum wheat, SSR, genetic diversity, botanical varieties*

INTRODUCTION

Durum wheat (*Triticum durum* Desf., 2n=4x==28; AABB) is the only tetraploid wheat, widely used today for human consumption, and the second most widely cultivated wheat specie in the world (Henkrar et al., 2016). It originated in the Fertile Crescent (10,000 BP) and spread over the northern side of the Mediterranean region (Moragues et al., 2006). Durum wheat is characterized by a high polymorphism and for the number of botanical varieties, ecological types and varieties is second only to bread wheat (Flyaksberger, 1938). As a result of the natural and human selection a variety of durum wheat landraces were developed and widely cultivated in many parts of the world until the middle of the 20th century. However, after the Green Revolution they were progressively replaced by the improved, genetically uniform cultivars, which lead to narrowing of their genetic base and loss of diversity. Nevertheless, scientists are convinced that the use of valuable local genetic resources is extremely important and may provide new alleles for the improvement of commercially valuable traits (Soriano et al., 2016).

In Azerbaijan cultivation of wheat has a long history. A wide range of soil and climatic conditions contributed to the development of rich vegetation, this in turn made possible to consider Azerbaijan as one of the most probable centers of wheat origin (Vavilov, 1967). Durum wheat varieties of Azerbaijan - Sary bughda, Gara bughda, Agh bughda, Gyrmizy bughda were mentioned in references since the XIX century (Абдуллаев, 1957). As a result of breeding activities during 1980s years new durum wheat varieties such as Tartar, Vugar, Barakatli-95 were also created and registered by academician Jalal Aliyev (Əliyev, 1989). In addition, systematic activities have been conducted on collecting and conservation of botanical varieties and populations of different wheat species, as well as *T. durum* widespread in different regions of Azerbaijan. Currently, more than 2,000 wheat accessions are maintained in the National Genebank. These accessions together with durum wheat varieties represent a particularly important group of genetic resources that can be used in breeding programmes in order to increase the available genetic variation, in terms of adaptation to different environments, end-product quality and resistance to diseases.

Knowledge of genetic diversity is crucial for understanding the relationships between cultivars and facilitating their use in new breeding strategies and crosses (Soriano et al., 2016). Molecular markers play a pivotal role in evaluation of genetic diversity (Henkrar et al., 2016). Although several markers have been used for analysis of genetic diversity and variety identification of durum wheat (Chen et al., 1994), microsatellite (SSR) markers proved to be more powerful due to abundance, allele richness, polymorphism and codominance. A number of studies confirmed the usefulness of SSR markers for evaluating the genetic diversity (Royo et al., 2010; Ruiz et al., 2012) and genetic mapping (Yousefi Javan et al., 2011) in different wheat species, as well as in *T. durum*. To date, only few studies have examined the genetic diversity and rela-

tionship among durum wheat acessions of Azerbaijan using RAPD, ISSR and SNP markers (Aliyev et al., 2007; Гаджиев и др., 2015; Abbasov et al., 2017). No work has been focused to study microsatellite diversity in Azerbaijani durum wheat.

The aim of the current study was to evaluate the diversity existing in a durum wheat collection of Azerbaijan using SSR markers and to determine genetic distances among different cultivars and botanical varieties.

MATERIALS AND METHODS

A total of one hundred forty-five accessions of *T. durum* Desf. belonged to 29 botanical varieties were used as a research material. Out of 145 accessions 36 were varieties created in different years.

Genomic DNA isolation was conducted following a CTAB protocol. Thirteen polymorphic SSR markers were used in the study (table 1). The Polymerase Chain Reactions (PCRs) were performed using fluorescent-dye labeled primers was as follows: initial denaturation at 95°C for 3 min; 40 cycles of denaturation at 95°C for 1 min, annealing at 50°C for 1 min and elongation at 72°C for 2 min; final elongation at 72°C for 10 min. Amplified DNA products were separated on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientifics) (Chao et al., 2007). Fragment analysis and allele calling were performed using GeneMapper software v.3.7 (Applied Biosystems).

PowerMarker v. 3.51 (Liu and Muse, 2005) software was used to calculate a total number of alleles, expected heterozigosity (He), observed heterozigosity (Ho), polymorphism information content (PIC). Allele frequencies and distances based on frequencies among different botanical varieties were also calculated using PowerMarker. Cluster analysis, PCoA analysis and Neighbour-joining tree was undertaken using software package DARwin 6.0 (Perrier and Jacquemoud-Collet, 2006).

RESULTS AND DISCUSSION

Exploiting the variability of wheat landraces requires previous knowledge of their genetic diversity.

Genetic diversity and relationships among 145 durum wheat accessions belonged to 29 botanical varieties, including 36 varieties was studied using simple sequence repeat (SSR) marker system. The variation of genetic diversity and allele distribution were strongly dependent on the analyzed loci. A total of 104 amplicons were produced using 13 SSR primers for 145 durum wheat accessions, with an average of 8 bands per primer (table 1). The number of alleles generated by each primer varied from 2 for barc182 to 15 for primer pair barc147. Ten out of 13 primers produced more than 5 alleles. High number of alleles observed in our experiments can be explained by the unique mechanism responsible for generating SSR allelic diversity by replication slippage (Al-Faifi et al., 2016). Similar observations were also reported by Mangini et al. (2008). The observed heterozygosity was low for the collection (H_O =0.07) and varied from 0.00 to 0.42 with maximum value obtained for primer pair gwm361. The low observed heterozygosity level is probably due to the self-pollinated nature of the durum wheat. Major allele frequency, which is inversely proportional to the gene diversity, ranged from 0.21 to 0.93 and averaged 0.51. The main diversity parameters of the locus, the expected heterozygosity (H_E) and the polymorphism information content (PIC) had a wide range. The primer pairs with the maximum value of major allele frequency showed the minimum diversity indices. Among the 13 loci maximum gene diversity (H_E=0.87) and PIC (0.86) values were obtained for locus *barc174*, while loci *barc1021* and *barc182* had minimum H_E and PIC. The majority of the markers, with PIC <0.50, were moderately to highly informative, according to the criteria proposed by Botstein et al. (1980). The mean H_E and PIC values considering all accessions of *T. durum* were 0.62 and 0.58, respectively. The results indicate high diversity in the studied durum wheat collection. The results are higher than the one reported by Carvalho et al. (2009). In his study of 51 durum wheat varieties of Portugal origin, belonging to 26 different botanical species, Carvalho and his colleagues revealed a low level of polymorphism.

Table 1. Summary statistics of the 13 SSR markers used in the study

Marker	Allele No	Major Allele Freq.	H_O	H_E	PIC
barc17	7	0.49	0.03	0.66	0.61
barc212	13	0.35	0.06	0.80	0.77
barc1021	3	0.93	0.00	0.13	0.12
barc117	5	0.54	0.03	0.57	0.49
barc113	8	0.43	0.02	0.68	0.63
gwm332	6	0.37	0.07	0.73	0.68
barc174	8	0.65	0.02	0.54	0.50
barc200	8	0.62	0.19	0.58	0.56
barc147	15	0.21	0.03	0.87	0.86
barc163	11	0.28	0.00	0.82	0.80
barc74	10	0.27	0.07	0.81	0.78
gwm361	8	0.53	0.42	0.67	0.65
barc182	2	0.92	0.00	0.14	0.13
Mean	**8**	**0.51**	**0.07**	**0.62**	**0.58**
Total	**104**				

Table 2. Summary statistics for different subsets of *T. durum* accessions

Subsets	Sample size	Allele No	Major allele frequency	H_O	H_E	PIC
var. ***affine***	**7**	**3.4**	**0.58**	**0.03**	**0.52**	**0.48**
var. *africanum*	1	1.0	0.88	0.00	0.00	0.11
var. ***alboobscurum***	**2**	**1.5**	**0.81**	**0.04**	**0.20**	**0.16**
var. ***alboprovinciale***	**3**	**2.3**	**0.58**	**0.10**	**0.49**	**0.40**
var. *alexsandrinum*	1	1.2	0.88	0.23	0.12	0.09
var. ***apulicum***	**10**	**2.9**	**0.67**	**0.07**	**0.42**	**0.38**
var. *areichenbachii*	2	1.3	0.81	0.00	0.00	0.18
var. *boeufii*	2	1.8	0.62	0.04	0.39	0.30
var. ***caerulescens***	**4**	**2.4**	**0.60**	**0.06**	**0.46**	**0.39**
var. ***erythromelan***	**5**	**3.2**	**0.56**	**0.05**	**0.54**	**0.49**
var. *horanomelanopus*	1	1.2	0.88	0.23	0.12	0.09
var. ***hordeiforme***	**15**	**4.3**	**0.57**	**0.08**	**0.55**	**0.51**
var. ***leucomelan***	**9**	**2.9**	**0.59**	**0.08**	**0.50**	**0.44**
var. ***leucurum***	**20**	**5.0**	**0.52**	**0.12**	**0.61**	**0.57**
var. *lybicum*	1	1.1	0.96	0.08	0.04	0.03
var. *melano leucurum*	2	1.7	0.69	0.04	0.32	0.24
var. ***melanopus***	**13**	**3.8**	**0.59**	**0.07**	**0.52**	**0.48**
var. *melanopus turgidoid*	1	0.9	0.81	0.00	0.00	0.18
var. ***murciense***	**6**	**3.1**	**0.57**	**0.04**	**0.51**	**0.46**
var. *mutico affine*	1	1.0	1.00	0.00	0.00	0.00
var. *mutico africanum*	1	1.0	0.88	0.00	0.00	0.11
var. *mutico coerulescens*	1	0.9	0.92	0.00	0.00	0.08
var. *mutico hordeiforme*	2	1.6	0.73	0.12	0.28	0.22
var. *mutico leucurum*	1	0.8	0.65	0.00	0.00	0.34
var. *mutico lybicum*	1	0.8	0.85	0.00	0.00	0.15
var. *muticoobscurum*	1	0.8	0.77	0.00	0.00	0.23
var. ***niloticum***	**7**	**2.6**	**0.70**	**0.05**	**0.39**	**0.35**
var. ***obscurum***	**8**	**3.2**	**0.59**	**0.09**	**0.52**	**0.47**
var. *reichenbachii*	1	0.8	0.85	0.00	0.00	0.15

Among studied botanical varieties, the number of total alleles was associated with the sample size. So, var. *leucurum* and var. *hordeiforme* with the highest sample sizes had the maximum allele numbers as well (5 and 4.3, respectively) (table 2). The higher diversities were obtained for var. *leucurum* (PIC=0.57) and var. *hordeiforme* (PIC=0.51), followed by 5 accessions of var. *erythromelan*. It is interesting to note that, var. a*lboprovinciale* and var. *caerulescens* represented by only 3 and 4 genotypes, exhibited higher gene diversity (H_E=0.49 and 0.46). On the contrary, var. *apulicum* with 10 genotypes had relatively lower indices (H_E=0.42; PIC=0.38).

Genetic diversity indices, taking into account the different subgroups (varieties and accessions of different botanical varieties) were little bit lower for varieties (H_E=0.58) in compare with genebank accessions (H_E=0.62). This indicates that the genetic diversity in tetraploid durum wheat was only slightly narrowed down during the second part of the 20th century due to the breeding.

A disimilarity matrix based on the SSR fragments was used to establish the level of relatedness among durum wheat botanical varieties and varieties. Pair-wise estimates of dissimilarity coefficient (GD) ranged from 0.00 to 1.0 with an average of 0.60. 100% similarity observed for some genotype pairs. Eight main clusters were detected in the dendrogram generated based on SSR dissimilarity matrix (figure 1).

No clear grouping pattern was revealed based on botanical varieties. All clusters were quite diverse containing from 1 to 4 genotypes of several botanical varieties, with some exceptions. This fact indicates the significant amount of shared alleles among different botanical varieties of *T. durum*. Moreover, the studied durum wheat accessions were all collected from one country - Azerbaijan. The relatively narrow collection area (in compare with the germplasm collected from different countries) of samples and thus the similar soil-climatic condition and similar genetic background may also reflect their mixed grouping in the dendrogram. Thus, the low genetic distance estimated between botanical varieties may be explained by geographic proximity. On the other hand, the dissimilarity indices within clusters were also high, indicating high diversity of the studied *T. durum* genotypes.

However, some tendencies were recorded on the grouping of genotypes according to the botanical varieties. Out of 10 genotypes of var. *apulicum* (red colour) 7 fell into cluster VIII.

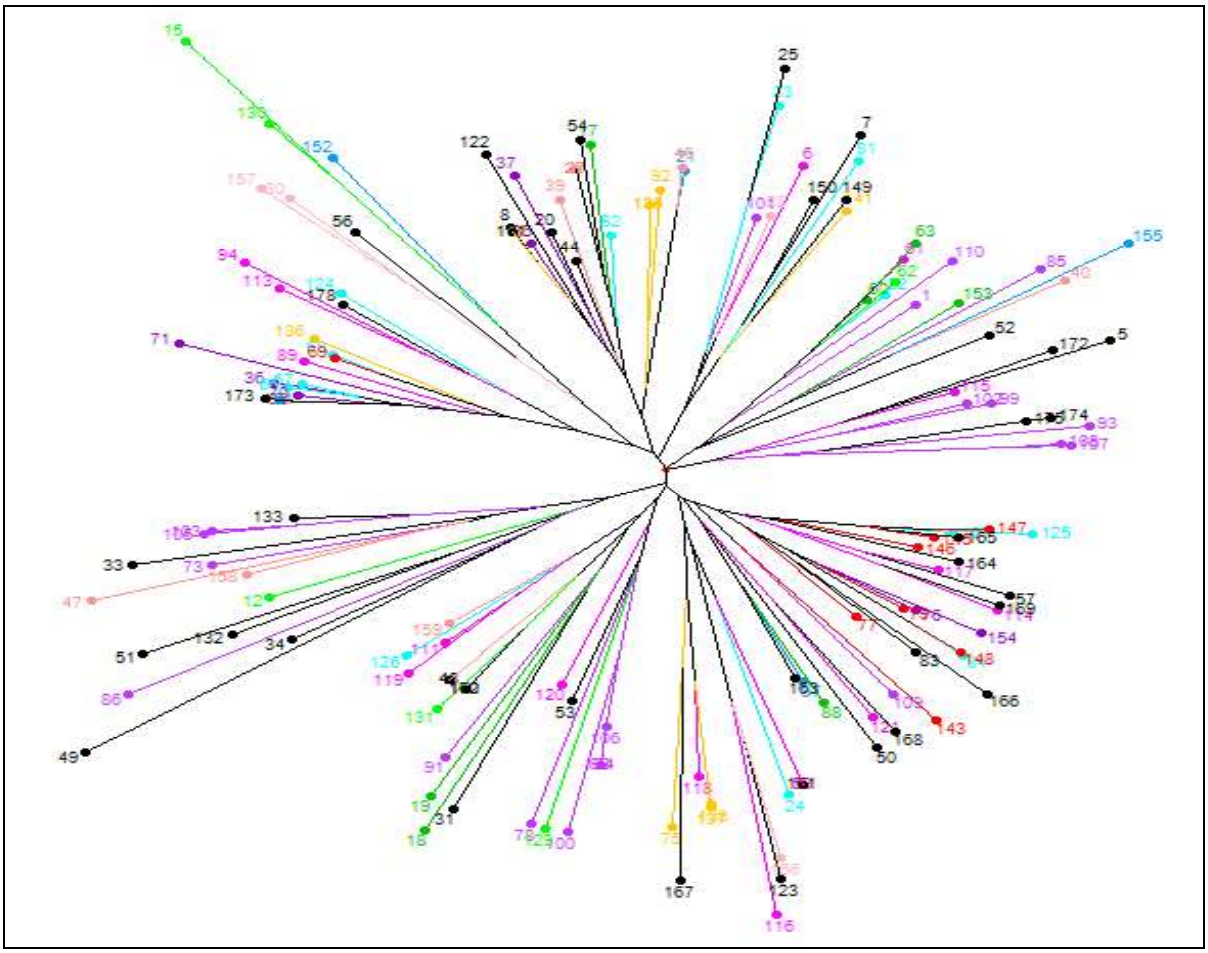

Figure 1. Dendrogram showing genetic relationship among 145 *T. durum* L. accessions based on Nei's genetic distance. Different colors indicate different botanical varieties.

Twenty var. *leucurum* genotypes (violet colour) unequally distributed among clusters; 6 of them were placed very closely in cluster VII, 5 in cluster I and 4 in cluster VI and formed independent groups. Similar grouping pattern was noted for 15 accessions of var. *hordeiforme* (pink colour), 6 of which grouped in cluster VIII and for 9 accessions of var. *leucomelan* (yellow colour); 4 of them were in cluster IV and 3 in cluster VIII with 100% similarity between some pairs. The most diverse cluster in terms of botanical varieties was clusters II and III.

Nei's genetic distance between varieties and genebank accessions was very low (GD=0.062). In a number of studies durum wheat landraces are considered as a source of variability that able to provide new favorable alleles to be introgressed into modern cultivars (Lopez et al., 2015). The low genetic distance between varieties and landraces in the current study shows the effective use of durum landraces by durum wheat breeding programmes.

Among studied 36 improved cultivars Sharg and Jafari had same alleles for all 13 SSR loci and thus showed 100% similarity. This was in accordance with Abbasov et al. (2017). The results of the cluster analysis based on 1058 SNPs obtained from GBS analysis confirmed the high similarity between Sharg and Jafari (Abbasov et al. 2017).

In addition, Mugan variety exhibited 100% similarity with genotype from var. *leucomelan*, Savalan with var. *erythromelan* and Barakatly 95 with var. *hordeiforme*. Varieties Vugar and Shiraslan 23 were closer to each other, as well.

PCoA analysis confirmed subgrouping obtained by cluster analysis and showed intermixing of studied botanical varieties across the coordinates (figure 2). The first five axes explained 29.4 % of cumulative variation.

All genotypes were distributed along the scatter plot and did not form genetically well differentiated groups.

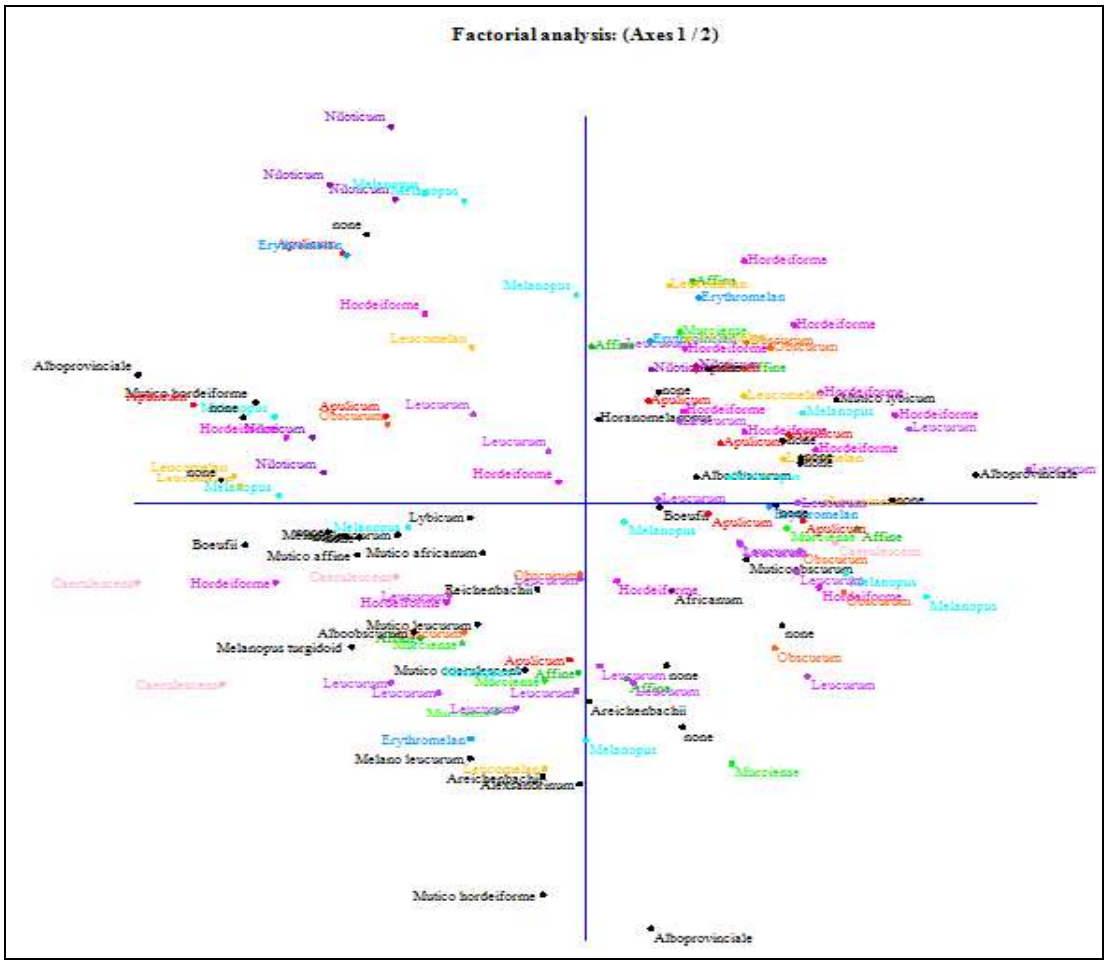

Figure 2. Scatter plot of 145 *T. durum* accessions using 13 SSR markers. Different colors indicate different botanical varieties.

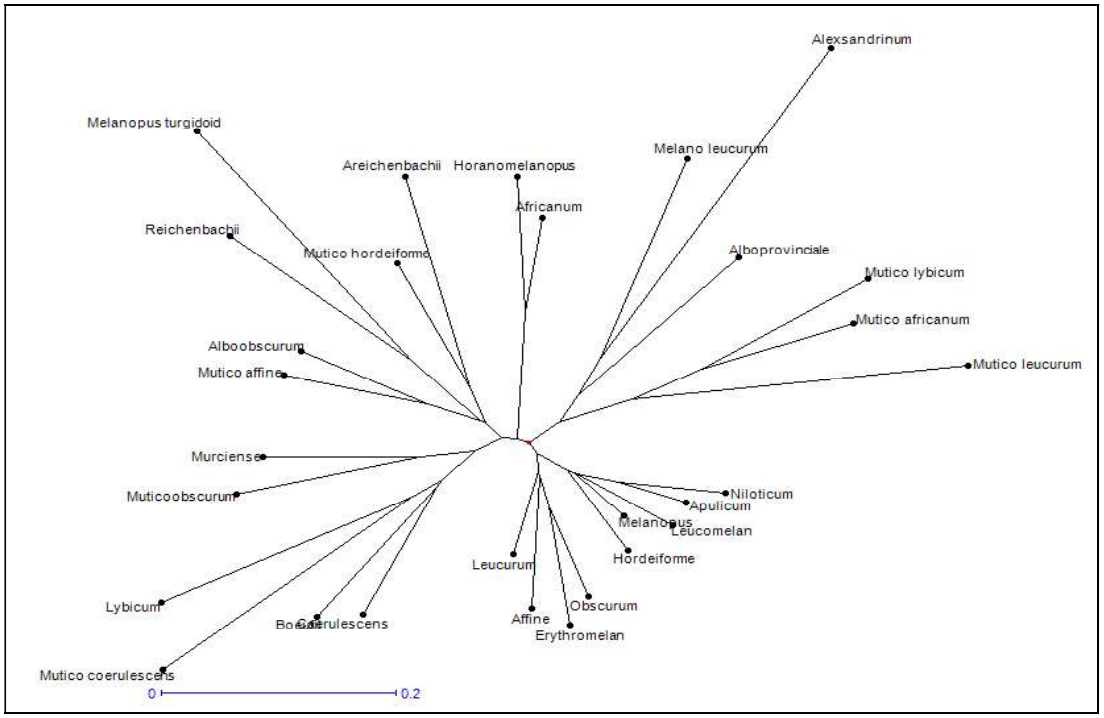

Figure 3. Dendrogram of *T. durum* botanical varieties based on Nei's genetic distance.

Genetic dissimilarity (GD) index among different botanical varieties ranged from 0.13 to 0.86, with mean value of 0.47. The highest similarity was noted between var. *hordeiforme* and var. *melonopus*, while var. *leucurum* and var. *melanopus turgidoid* were the most distant. Similarly, Hajiyev et al. (2015) recorded close relationship among mentioned botanical varieties. In another study of durum wheat accessions belonged to 13 botanical varieties Abbasov and his colleagues reveled high similarity within var. *leucurum*, followed by var. *hordeiforme* and var. *leucomelan*; they formed separate groups with minimal distances among them (Abbasov et al., 2017).

To visually demonstrate a dendrogram was created based on allele frequencies where 3 main clusters were further divided into several subclusters (figure 3). Var. *leucurum*, var. *affine*, var. *erythromelan* and var. *obscurum* formed a separate subcluster within cluster I, indicating their similar genetic background. The second subcluster contained closely located botanical varieties var. *hordeiforme*, var. *melanopus*, var. *leucomelan*, var. *apulicum* and var. *niloticum* with dissimilarity indices between 0.13 and 0.20.

Among the rest botanical varieties var. *carulescens* and var. *boefii* (GD=0.29); var. *murciense* and var. *muticoobscurum* (GD=0.29); var. *alboobscurum* and var. *muticoaffine* (GD=0.24) were closer to each other.

So, the present study shows that SSR markers can be used effectively to estimate genetic diversity and relationship among durum wheat genotypes. The SSR technique confirmed the existence of rich diversity in *T. durum* cultivars and botanical varieties of Azerbaijan origin. The studied collection of 145 durum wheat varieties has rich genetic diversity, which is reflected in high values of allele number, expected heterozygosity and PIC. Along with that, they share alleles among themselves, which make impossible their clear differentiating to the clusters. The valuable diversity revealed in the current collection can be used as a source for future breeding programmes.

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SSR Markerlərlə Azərbaycan Mənşəli Bərk Buğda Kolleksiyasında Genetik Müxtəlifliyin Qiymətləndirilməsi

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29 növmüxtəlifliyinə aid bərk buğda (*T. durum* Desf.) sort və nümunələrində genetik müxtəliflik və genetik əlaqə SSR markerləri ilə tədqiq edilmişdir. Ümumilikdə, 13 SSR praymeri ilə 145 bərk buğda nümunəsi üçün 104 allel sintez olunmuş, hər praymerə düşən allel sayı 8 ədəd təşkil etmişdir. Gözlənilən heteroziqotluq (H_E) və polimorfizm informasiya tutumu (PIC) parametrlərinin orta göstəricisi, müvafiq olaraq, 0.62 və 0.58 vahid təşkil etmişdir ki, bu da kolleksiyada yüksək genetik müxtəlifliyin olduğunu göstərir. Növmüxtəliflikləri arasında ən çox müxtəliflik var. *leucurum* (PIC=0.57) və var. *hordeiforme* (PIC=0.51) nümunələri üçün qeydə alınmışdır. Dendroqramda bəzi istisnalar olmaqla, növmüxtəlifliklərinin aydın şəkildə qruplaşması müşahidə olunmamış, növmüxtəlifliklərinin əhəmiyyətli dərəcədə ortaq allellərə malik olduğu müəyyən edilmişdir. Var. *hordeiforme* və var. *melonopus* növmüxtəlifliklərinin bir-birinə ən yaxın, var. *leucurum* və var. *melanopus turgidoid* növmüxtəlifliklərinin isə ən uzaq olduğu aşkar edilmişdir. Bərk buğda kolleksiyasında aşkar edilmiş zəngin müxtəliflik qiymətli mənbə kimi gələcək seleksiya işlərində istifadə oluna bilər.

***Açar sözlər**: Bərk buğda, SSR, genetik müxtəliflik, növmüxtəlifliyi*

Изучение Генетическое Разнообразия Коллекции Азербайджанской Твердой Пшеницы на Основе SSR Маркеров

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С помощью SSR маркеров изучено генетическое разнообразие и генетическая связь между сортами и образцами твердой пшеницы (*T. durum* Desf.), принадлежащими к 29 разновидностям. В общей сложности, для 145 образцов твердой пшеницы с применением 13 SSR праймеров были синтезированы 104 аллели при среднем значении 8 аллелей на локус. Средние значения ожидаемой (H_O) гетерозиготности и величины информационного полиморфизма (PIC) составили 0,62 и 0,58 соответственно, что указывает на большое разнообразие в изученной коллекции. Наибольшее генетическое разнообразие было выявлено для разновидностей var. *leucurum* (PIC=0,57) и var. *hordeiforme* (PIC = 0,51). За некоторыми исключениями, на дендрограмме не обнаружено четкой группировки разновидностей и установлено, что разновидности обладают значительным количеством общих аллелей. Наибольшее генетическое сходство отмечено между разновидностями var. *hordeiforme* и var. *melonopus*, в то время как var. *leucurum* и var. *melanopus turgidoid* оказались самыми отдаленными. Выявленное среди коллекции твердой пшеницы богатое генетическое разнообразие может быть использовано в качестве ценного источника для будущих селекционных программ.

Ключевые слова: *Твердая пшеница, SSR, генетическое разнообразие, разновидности*