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Genetics**STUDY OF GENETIC DIVERSITY IN BREAD WHEAT (*T.AESTIVUM* L.) VARIETIES AND COLLECTION ACCESSIONS CONSERVED IN NATIONAL GENE BANK USING MOLECULAR MARKERS****E.S.Hajiyev¹, Corresponding Member of NAS of Azerbaijan Z.İ.Akparov¹, M.A.Abbasov^{1,2}, S.M.Babayeva¹, S.V.Saidova¹, R.Ch.Sharma³**

*Twelve bread wheat (*T. aestivum* L.) varieties and 98 accessions belonged to 11 botanical varieties were assessed using ISSR markers. Eight of 10 initially tested ISSR markers which had a high polymorphism level were used to study genetic diversity. Ninety seven polymorphic bands were obtained as a result of molecular analysis. Genotypes were grouped in 12 clusters in the dendrogram created using PAST statistic program. Analyses of the results revealed that accessions of same botanical varieties were placed in the same clusters and were genetically close to each other. Genetically distant genotypes can be used as an initial material in future breeding process. Primers UBC841 (0.96), UBC857 (0.98), UBC864 (0.98) and UBC873 (0.96) were recommended as effective primers to study genetic variation in bread wheat genotypes. As a result of study, for the first time, core collection consisted of a set of 20 accessions was created, that combines genetic diversity of 110 accessions. Selected accessions can be used as an initial material in creation of new varieties.*

Keywords: *T. aestivum* L., botanical variety, genetic diversity, ISSR markers, dendrogram, core collection

Introduction

A genetic marker is an allele, band or trait that can be used to identify and classify individuals or differentiate between individuals in a population [5, 7, 8]. It can be divided into morphological, protein-based and DNA-based markers. All three types are widely used in evaluation of genetic diversity of wheat [4, 12]. In wheat in comparison with other cereals morphological traits have been thoroughly researched and a large number of landraces and botanical varieties were determined on the basis of some main morphological markers (spike, seed and awn color, hairy spike). For many years these traits had an important role in wheat breeding and used by breeders or plant researchers to identify varieties and landraces. At present, these features are essential in evaluation of collection accessions and selection of primary material for breeding. It should be noted that the study of botanical varietal traits for the first time was started in Germany and later developed by Vavilov and his colleagues. Azerbaijani scientist Mirza Gokgol's merit in characterization of wheats according to botanical varietal traits was great and several new botanical varieties were first determined un-

der his authorship. Currently more than 80 botanical varieties of bread wheat were recorded [2, 3].

With the application of protein and molecular markers, a new generation of the markers has been introduced in assessment of wheat genetic diversity [5, 12]. By analyzing wheat varieties based on gliadin and glutenin markers a number of allele component blocks were identified and their linkage with different traits was determined. The next advance in genetic markers was the advent of DNA-based markers, which has revolutionized the entire scenario of biological sciences. DNA markers provide a more direct and efficient tool for evaluation, characterization and genetic passportization of plant collections [1, 6, 9, 11]. In contrast to morphological and protein markers DNA markers have many advantages. They are insensitive to environment, highly polymorphic, abundant and more reliable. Moreover, analysis can be conducted at any stage of plant growth. Popular molecular markers for diversity analysis are RFLP, RAPD, SSR, ISSR, AFLP, SRAP etc [13, 14]. Among various PCR-based markers ISSRs are relatively simple and rapid. In addi-

tion, no prior knowledge about the sequence under the investigation is required. The objective of this study was to evaluate genetic diversity among bread wheat accessions and botanical varieties using ISSR markers.

Material and methods

Twelve bread wheat (*T.aestivum* L.) varieties and 98 accessions belonged to different botanical varieties were used for molecular-genetic studies. DNA extraction was performed according to the CTAB (cetyltrimetilammonium bromide) protocol proposed by Doyle & Doyle [6] with some modifications. The concentration and purity of the DNA molecule was 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 (Applied Biosystems, USA). The name and sequences of used ISSR primers were presented in table 2. Electrophoresis of PCR products was per-

formed on 2% agarose gel by adding ethidium bromide and visualized under UV light using gel documenting system BioRad.

Statistical data analysis

Analysis of amplified fragments was performed using the computer program PAST. 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 [15]. Construction of the dendrogram and evaluation of genetic similarity between the sample pairs was performed according to Nei's genetic distance index (1987) [10], using PAST program.

Results and discussion

Ten ISSR primers were prescreened and 8 primer sets that produced clear and unambiguous bands were selected for further analysis of 110 bread wheat accessions. The main area of distribution of amplicons was located in the range of 100-2200 bp. A total of 136 reliable fragments were amplified for studied collection, of which 97 were polymorphic. The highest number of bands were noted for UBC841 (20) and UBC864 (22) (table 1). The average number of fragments per primer was 17 and the average number of polymorphic bands was 12.1.

Table 1

Genetic parameters of used ISSR primers in bread wheat collection

Primer	Sequence (5'-3')	Number of bands	Number of polymorphic bands	Polymorphism %	Genetic Diversity Index
UBC112	(GA) ₄ (CA) ₄	13	10	77	0.92
UBC808	(AG) ₈ C	18	13	72	0.94
UBC811	(GA) ₈ C	19	10	53	0.69
UBC827	(AC) ₇ C	15	15	100	0.83
UBC841	G(ACA) ₄ (GC) ₃ C	20	13	65	0.96
UBC857	(AC) ₈ TT	19	14	74	0.98
UBC864	TAG(GT) ₆ GAA	22	13	59	0.98
UBC873	(GA) ₄ (CA) ₄	10	9	90	0.96
Total	-	136	97	-	-
Mean value	-	17	12.1	74	0.91

The percentage range of polymorphic bands was 53-100%, the mean value was 74%. In our experiment 100% polymorphism was only revealed by UBC827 containing (AC)₇C dinucleotide repeat motif, while UBC811 showed the lowest polymorphism. These results are consistent with literature. Thus, according to some researchers primers with (GA)_n dinucleotide repeat motifs demonstrate the low level of polymorphism [10]. Unique bands were obtained for some genotypes. A 800 bp fragment was amplified only in var. *barbarossa* genotype from Nakhchevan using primer UBC112, a 850 bp only in var. *milturum* from Devedchy using primer UBC841 and a 2200 bp fragment was noted only in var. *erythrosporum* from Abshehon using UBC857. UBC864 ISSR primer generated 3 different unique fragments for 3 different genotypes. Thus, fragments of 2000 bp, 150 bp and 1800 bp in length were noted in genotypes belonged to var. *erythrosporum* (Abshehon), var. *erythrosporum* (Oghuz) and var. *albidum* (Gobustan), respectively.

So, primers UBC 112, 841, 857 and 864 can be considered effective primers for identification of mentioned genotypes.

Genetic diversity indices (GDI), which reflect allele diversity and frequency was calculated for each primer and within botanical varieties. GDI values ranged from 0.69 (UBC 811) to 0.98, with a mean of 0.91. The maximum value of GD index was observed for primers UBC857 and UBC864. Among the primers used in the

present study UBC873 was highly informative and distinguished by both the highest GDI value (0.96) and polymorphism rate (90%). The high genetic diversity index in studied collection could be explained by the fact that the studied genotypes were collected from different regions of Azerbaijan with diverse and rich soil and climate conditions.

The calculation of Nei's genetic diversity index within botanical varieties revealed that the genetic diversity within var. *erythrosporum* represented by 20 accessions was higher (0.90). In spite of little number of genotypes (5) var. *velutinum* also showed higher (0.77) genetic diversity (Fig. 1). Hierarchical cluster analysis (UPGMA) is performed to study genetic relationship among genotypes. Twelve clusters were revealed in the dendrogram resulting from the eight primers data (Fig. 2). The grouping of samples in clusters was complex and mixed. Five of studied accessions formed separate clusters as genetically divergent forms. In addition, the existence of some particularly different accessions within each major cluster led to the formation of subclusters containing only a single or few samples. Cluster V contained 30.9% of total accessions, among which prevailed genotypes of var. *lutescens* (10) and var. *gracum* (7). The most closely related genotypes in this group were var. *gracum* collected from Gobustan and var. *lutescens* collected from Terter (0.12). The lowest relationship was recorded for genotypes var. *albidum* (Terter) and var. *gracum* (Abshehon) (0.90).

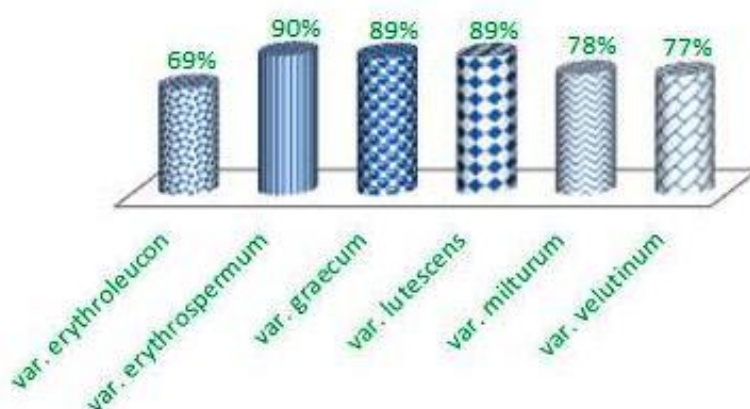


Fig. 1. Genetic diversity index within some botanical varieties

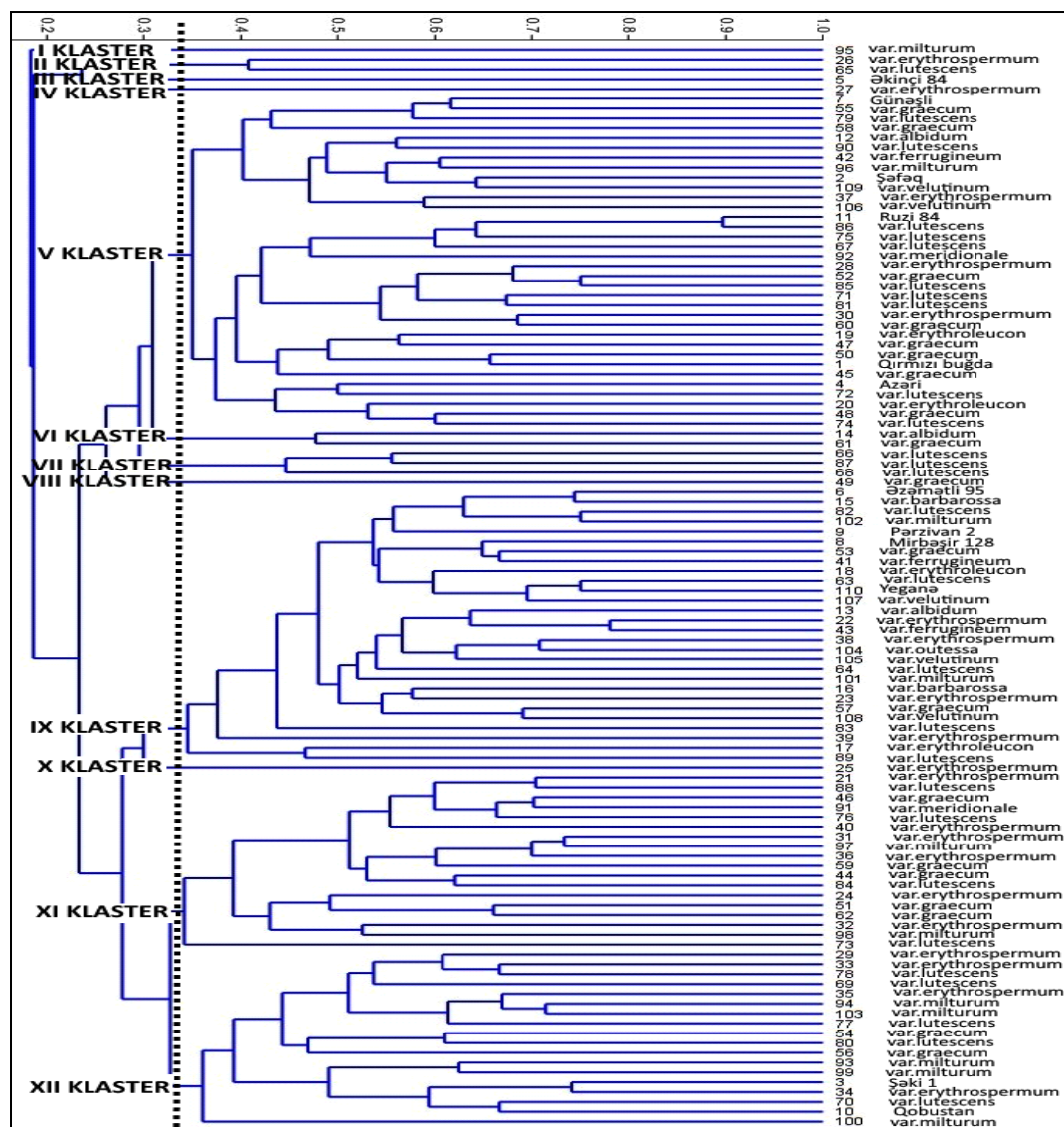


Fig. 2. Dendrogram showing genetic relationship among 110 bread wheat genotypes

Cluster IX involved 25.5% of total genotypes, including both botanical varieties and released varieties (Azamatly 95, Parzivan 2, Mirbashir 128, Yegana). Four genotypes of *var. erythrosperrum* and 4 genotypes of *var. lutescens* occurred in this cluster. The least genetic distance (0.18) was noted between genotypes 39 (*var. erythrosperrum*) and 89 (*var. lutescens*). The most distant (0.78) genotypes were 22 (*var. erythrosperrum*) and 43 (*var. ferrugineum*).

Each of clusters XI and XII consisted of 18 genotypes. Cluster XI contained mainly accessions of *var. erythrosperrum* and XII cluster

var. miltirum. One genotype in each cluster (respectively, *var. lutescens* (Absheron) and *var. miltirum* (Baku) formed a separate subcluster.

As it was noted, Akinchy 84, *var. erythrosperrum* (Absheron), *var. miltirum* (Absheron), *var. graecum* (Gobustan) and *var. miltirum* (Baku) having completely different profile among 110 genotypes, formed a separate cluster and estimated as genetically distinct genotypes.

Another objective of the research was the establishment of core collection. Based on the results 20 most distant genotypes representing genetic diversity of 110 accessions were selected (Table 2).

Table 2

The list of accessions of core collection

№	Code	Botanical variety	Collected region	№	Code	Botanical variety	Collected region
1	6276	<i>var.meridionale</i>	Absheron	11	6284	<i>var.erythroleucon</i>	Absheron
2	6280	<i>var.lutescens</i>	Absheron	12	6268	<i>var.erythrosporum</i>	Sheki
3	6286	<i>var.erythroleucon</i>	Absheron	13	9528	<i>var.erythrosporum</i>	Berde
4	6298	<i>var.erythrosporum</i>	Absheron	14	6294	<i>var.graecum</i>	Berde
5	6350	<i>var.lutescens</i>	Terter	15	7040	<i>var.graecum</i>	Gobustan
6	6929	<i>var.graecum</i>	Lerik	16	6169	<i>var.lutescens</i>	Terter
7	6932	<i>var.milturum</i>	Shamakhy	17	7042	<i>var.lutescens</i>	Gobustan
8	7038	<i>var.graecum</i>	Gobustan	18	7049	<i>var.lutescens</i>	Gobustan
9	7046	<i>var.albidum</i>	Gobustan	19	7247	<i>var.milturum</i>	Masally
10	7321	<i>var.barbarossa</i>	Absheron	20	8738	<i>var.velutinum</i>	Absheron

This collection can be used as an initial material for breeding. Genetically divergent genotypes with better agricultural traits selected from core collection can be crossed to obtain valuable hybrids for breeding.

This study confirms the existence of significant genetic variation among bread wheat varieties and accessions. This result is expected and desirable. The results also showed that ISSR markers could be successfully used in genetic diversity assessment of bread wheat collection. Knowledge of genetic variability among wheat genotypes will allow selection of appropriate parents for crossing.

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MILLİ GENBANKDA SAXLANILAN YUMŞAQ BUĞDANIN (*T.AESTIVUM* L.) SORT VƏ KOLLEKSIYA NÜMUNƏLƏRİNDƏ GENETİK MÜXTƏLİFLİYİN MOLEKULYAR MARKERLƏRLƏ TƏDQIQI

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Tədqiqat işində yumşaq buğdanın (*T. aestivum* L.) 12 sortu və 11 növmüxtəlifliyinə aid 98 kolleksiya nümunəsi ISSR markerləri ilə qiymətləndirilmişdir. İlk olaraq yoxlanılmış 10 ISSR markerindən 8-i yüksək polimorfizmə malik olduğundan nümunələrin genetik müxtəlifliyi bu praymerlərlə tədqiq edilmişdir. Molekulyar analizlər nəticəsində 97 polimorf bənd alınmışdır. PAST statistik proqramı ilə Cakkard oxşarlıq indeksi əsasında qurulmuş dendroqramda genotiplər 12 klasterdə qruplaşmışdır. Nəticələrin təhlili zamanı məlum olmuşdur ki, eyni növmüxtəliflik əlamətlərinə malik olan nümunələr eyni klasterdə yerləşməklə genetik cəhətdən bir-birinə yaxın olmuşdur. Tədqiqatın nəticələrinə əsasən genetik cəhətdən uzaq olan genotiplərdən gələcəkdə seleksiya prosesində başlanğıc material kimi istifadə etmək olar. UBC841 (0.96), UBC857 (0.98), UBC864 (0.98) və UBC873 (0.96) praymerləri üçün hesablanmış genetik müxtəliflik indeksi yüksək olduğundan, onlar yumşaq buğda genotiplərində genetik variasiyanın öyrənilməsində polimorf praymerlər kimi tövsiyə edilir. Tədqiqatın nəticəsində, ilk dəfə olaraq, Azərbaycanda 20 nümunədən ibarət özək kolleksiya yaradılmışdır ki, bu nümunələr özündə 110 nümunənin genetik müxtəlifliyini birləşdirir. Seçilən nümunələrdən yeni sortların yaradılmasında başlanğıc material kimi istifadə etmək olar.

Açar sözlər: *T.aestivum* L., növmüxtəlifliyi, genetik müxtəliflik, ISSR markerlər, dendroqram, özək kolleksiya

ИЗУЧЕНИЕ ГЕНЕТИЧЕСКОГО РАЗНООБРАЗИЯ СОХРАНЯЕМЫХ В НАЦИОНАЛЬНОМ ГЕНБАНКЕ СОРТОВ И КОЛЛЕКЦИОННЫХ МАТЕРИАЛОВ МЯГКОЙ ПШЕНИЦЫ (*T.AESTIVUM* L.) С ИСПОЛЬЗОВАНИЕМ МОЛЕКУЛЯРНЫХ МАРКЕРОВ

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В настоящем исследовании приведены результаты оценки 12 сортов и 98 коллекционных материалов 11-ти разновидностей мягкой пшеницы (*T. aestivum* L.) на основе ISSR анализа. 8 из 10 протестированных праймеров, проявляя высокий уровень полиморфизма, были включены в анализ для оценки генетического разнообразия генотипов. В результате молекулярного анализа были получены 97 полиморфных фрагментов. Дендрограмма, построенная с помощью статистической программы PAST и на основе индекса генетического сходства Джаккарда, сгруппировала генотипы в 12 основных кластерах. В результате анализа полученных данных было установлено, что генотипы одной разновидности, объединенные в общем кластере, являются генетически близкими. Генетически отдаленные генотипы могут быть использованы в качестве исходного материала в дальнейших селекционных программах. Праймеры UBC841 (0.96), UBC857 (0.98), UBC864 (0.98) и UBC873 (0.96), для которых вычислен высокий индекс генетического разнообразия, могут быть рекомендованы как наиболее полиморфные для изучения генетической вариативности генотипов мягкой пшеницы. Впервые в Азербайджане в результате исследования была создана ко-коллекция, состоящая из 20 образцов мягкой пшеницы, которая объединяет в себе генетическое разнообразие 110 генотипов. Отобранные образцы могут быть использованы в качестве исходного материала для создания новых сортов.

Ключевые слова: *T.aestivum* L., разновидности, генетическое разнообразие, ISSR маркеры, дендрограмма, ко-коллекция