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GENETIC DIVERSITY OF SOME BREAD WHEAT GENOTYPES USING RAPD TECHNIQUE

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Abstract

A Group of five bread wheat genotypes that were newly introduced to Iraq were assessed for their genetic similarity using the random amplified polymorphic DNA (RAPD) technique. The genomic DNA of the genotypes was amplified with five RAPD primers. Used primers produced total amplified bands of 63 out of which 25 found to be polymorphic presenting polymorphism percentage of 39.7%. Amplified fragments per primer reached a maximum of 16 bands produced by primer OPF-10 resulting in 25% polymorphism. Using cluster analysis, the genotypes were distributed into two sub-clusters; the first (A) including a single genotype (G5), while the other 4 were grouped in the second sub-cluster (B) which divided into two subgroups. Similarity values showed G1 and G3 as the closest genotypes (97%), while G5 and G3 had the lowest value of similarity with 44%. Genotype G1 showed a unique band sized 3000bp using primer OPP-05, same band size appeared in G3 using OPA-06, while G5 had a unique band with size of 200bp using OPP-5. This study highlighted the genetic similarity among the five introduced genotypes showing their potential importance as promising genetic material for wheat breeding programs in Iraq to develop wheat germplasms considering further

genetic similarity investigation should be followed for the promising genotypes.

Keywords: RAPD, Wheat, Genetic diversity, Breeding, Genotype.

التباعد الوراثي لبعض التراكيب الوراثية من حنطة الخبز بأستخدام تقنية RAPD

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الخلاصة

تم تقييم مجموعة من خمسة تراكيب وراثية من حنطة الخبز مدخلة حديثاً الى العراق لتنوعها وتشابهها الوراثي بأستخدام تقنية الحمض النووي المتعدد الأشكال المضخم عشوائياً (RAPD). أستخدمت 5 بادئات RAPD عشوائية لتضاعف الحمض النووي الجينومي للتراكيب الوراثية الخمسة. أنتجت البادئات المستخدمة إجمالي حزم مضخمة قدرها 63 حزمة من بينها 25 حزمة متباينة أعطت نسبة تباين قدرها 39.7%. بلغ عدد الحزم المكوثرة لكل بادئ الى حد أقصى بلغ 16 حزمة أنتجت بواسطة البادئ OPF-10 مع نسبة تباين بلغت 25%. بأستخدام تحليل القرابة الوراثية، قسمت التراكيب الوراثية الى مجموعتين، أحتل التركيب الوراثي منفرداً مجموعة واحدة (A) في حين أجتمعت التراكيب الوراثية الأخرى في المجموعة الثانية (B) والتي أنقسمت الى مجوعتين فرعيتين. أظهرت قيم التشابه الوراثي أن G1 وG3 كانت أقرب التراكيب الوراثية وأكثرها تطابق بنسبة 97% في حين أعطى G5 وG3 نسبة تقارب بلغت 44%. أظهر التركيب الوراثي G1 حزمة فريدة بحجم 3000 زوج قاعدة بأستخدام البادئ OPP-05 وظهر نفس حجم الحزمة في G3 بأستخدام OPA-06، في حين أظهر G5 حزمة فريدة بحجم 200 بأستخدام البادئ OPP-05. لقد سلطت هذه الدراسة الضوء على التنوع الوراثي بين التراكيب الوراثية الخمسة المدخلة حيثا للعراق وأظهرت أهميتها كونها تراكيب وراثية واعدة لبرامج تربية الحنطة في العراق لتطوير التراكيب الوراثية للحنطة، مع الأخذ بنظر الأعتبار ضرورة إجراء المزيد من البحث عن التنوع الوراثي للتراكيب الوراثية الواعدة.

كلمات مفتاحية: الحنطة، التباعد الوراثي، التراكيب الوراثية، RAPD، تربية النبات.

Introduction

The genus Triticum includes 10 species, *Triticum. aestivum* which wheat is belonging to; is the most economic species. Bread wheat is classified as a hexaploidy self-pollinated crop having 42 chromosomes with approximately 17,000 Mb of large size genome (5). Polygenic traits of wheat such as seed yield are difficult to manage due to the instability under altered environmental condition(7, 14 and 15) No doubt wheat is one of the most important cereal crops worldwide and the main source of staple consumed food for human beings. Recent reports indicated that the demand for wheat was increased by more than 40% in 2020 over the last half of 1990s (13). According to the FAO (2021), the International Wheat Production Statics (IWPS) Iraq ranked 22 in the global wheat production. The rapid increase in the world population has pushed plant breeders to improve plant productivity and develop new varieties with an enhanced ability to adapt the climate change and unfavorable environmental conditions (10). Introducing new germplasms to be used in breeding programs is vital approach to generate genetic similarity and improve crops. There is a critical need to develop genotypes with enhanced yield, nutritional value and able to tolerate stresses and adapt to climate changing, hence, introduction is an important approach for sustainable agriculture practices (1 and 2)

Over the last decades, molecular breeding of wheat has gained increasing importance. Wheat like many other crop species, the first step to improve is to have a true assessment and evaluation of the available genetic materials using the most efficient tool we have including markers assisted selection (MAS). Database availability about the genetic relationship and germplasm similarity of the breeding materials is of a huge advantage to assist breeding program strategies to improve any crop (4). The introduction of MAS tool in breeding programs provided a valuable method to characteries the genetic materials of many crop species. For its efficiency and simplicity, RAPD markers have been used widely to evaluate the genetic similarity and fingerprinting genomes of wheat germplasms. In this study 5 bread wheat genotypes newly introduced to Iraq were investigated for their genetic similarity using RAPD markers to include them in ongoing breeding programs to improve local varieties.

Materials and Methods

Genetic similarity of five introduced wheat genotypes (named G1, G2, G3, G4 and G5) (Pedigree presented in table 1) was investigated in this study. Young flag leaf samples were collected from adult plants of each genotype. The samples were kept in triazole and then genomic DNA extraction toke place following standard procedure (16) using DNA extraction kit (Genmonic DNA Mini Kit / Plant - Geneaid Biotech Ltd.) following protocol provided with the kit. Laboratory work was done at the central laboratory of the College of Agriculture, University of Anbar.

Table 1: Pedigree of the five studied wheat genotypes.

No.	Genotype	Pedigree
1	G1	BAJ #1/3/TRCH/SRTU//KACHU CMSS10Y00030S-099Y-099M-099NJ-099NJ-12WGY-0B MXI14-15\M49IBWSN\12
2	G2	ATTILA*2/PBW65//TAM200/TUI/3/TRCH/SRTU//KACHU CMSS10Y00243S-099Y-099M-099NJ-099NJ-13WGY-0B MXI14-15\M49IBWSN\107
3	G3	TUKURU//BAV92/RAYON/6/NG8201/KAUZ/4/SHA7//PRL/VEE#6/3/FASAN/5/MILAN/KAUZ*2/7/PVNCMSS10Y00843T-099TOPM-099Y-099M-099NJ-099NJ-5WGY-0B MXI14-15\M49IBWSN\190
4	G4	FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ*2/5/KITE/6/PVN/7/TRCH/SRTU//KACHUCMSS10Y00871T-099TOPM-099Y-099M-099NJ-099NJ-10WGY-0B MXI14-15\M49IBWSN\204
5	G5	TRCH/SRTU//KACHU*2/3/KINGBIRD #1 CMSS10Y00880T-099TOPM-099Y-099M-099NJ-099NJ-6WGY-0B MXI14-15\M49IBWSN\212

RAPD analysis: Five oligonucleotides RAPD primers (Bioneer, Korea) were selected (Table 2) and PCR amplification protocol (Table 3) applied on PCR reaction mixture volume of 12.5 µl.

Table 2: Nucleotide sequence and GC % of the 5 used RAPD primers.

RAPD Primers	Sequence	GC (%)	Source
OPA-06	5′ GGTCCCTGAC 3′	70	(9)
OPP-05	5′ CCCCGGTAAC 3′	70	(9)
OPA-09	5′ GGGTAACGCC 3′	70	(9)
OPF-10	5′ GGGCCACTCA 3′	70	(9)
UBC638	5′ GCGGTGACTA 3′	60	(8)

The Reaction mixture contained reaction buffer (1.25 µl 10x), $MgCl_2$ (1.25 µl 25mM), dNTP (1.00 µl 2.5 mM), Taq DNA polymerase (0.125 µl), primer (0.5 µl), and genomic DNA (10 ng).

Table 3: Thermal cycle of the PCR amplification protocol.

Steps	Temperature (°C)	Time (min.:sec)
Initial denaturation	95	03:00
Denaturation	95	00:40
Annealing	42	01:40
Extension	72	02:30
Repeat steps 2 - 4 for 40 cycles		
Final extension	72	10:00
Hold	4	-

Agarose gel electrophoresis: Obtained products were analyzed by gel electrophoresis in 1.5% agarose/TBE gel. Gels were stained using ethidium bromide and visualized in the UV light chamber and observed using the computer program UVIPhotoMW provided with UV light chamber. O'GeneRuler DNA ladder mix 100-10000bp (Thermo Scientific™ SM1173, Product Code.11803983, concentration 0.1 µg μL^{-1}) was used as DNA marker premixed with 6X loading dye for direct loading on gel.

Data analysis: Obtained amplified RAPD bands were scored for each of the five primers as 0 for absence and 1 for presence. Using unweighted pair group method with arithmetic mean (UPGMA), data were entered in a binary character matrix and a

similarity coefficient was collected to generate phylogenetic tree (dendrogram) between tested genotypes. Genetic similarity was estimated according to Nei and Lei (1970) (11).

Results and Discussion

Molecular characterization using RAPD primers: The genomic DNA of the studied wheat genotypes was amplified using five primers (ten arbitrary base each) and produced a total of 63 loci (Table 4). Amplified DNA bands ranged between 5 bands produced by primer OPF-10 and 16 bands by primer OPA-09. Out of total of 63 amplified bands, 25 bands showed polymorphism. The highest percentage of polymorphism was recorded for the primer OPA-06 with 66.7% followed by OPP-05 that showing polymorphism percentage of 45.1%. On the other hand, the lowest polymorphism percentage was given by primer UBC-638 and it is worth mentioning that this primer was identified by (8) to be co-segregated with powdery mildew resistance in wheat. The highest primer efficiency (41.7%) and discriminating ability (83.3%) were recorded for primer OPA-06. Primer OPA-09 had the lowest efficiency percentage and discriminating ability reached 7.9% and 8%, respectively. In this study the highest percentage of polymorphism (66.7%) was obtained from OPA-06, while UBC638 exhibited the lowest percentage revealing 21.4%.

Table 4: Detected polymorphism of the five RAPD primers.

Primer	Total bands	Polymorphic-bands	Polymorphism %	Primer efficiency %	Discriminating ability %	Size bp
OPA-06	15	10	66.7	41.7	83.3	3000-500
OPP-05	13	6	45.1	20.6	24	2500-500
OPA-09	5	2	40	7.9	8	2000-550
OPF-10	16	4	25	25.4	16	3100-400
UBC638	14	3	21.4	22.2	12	2000-450
Total	63	25	39.7			

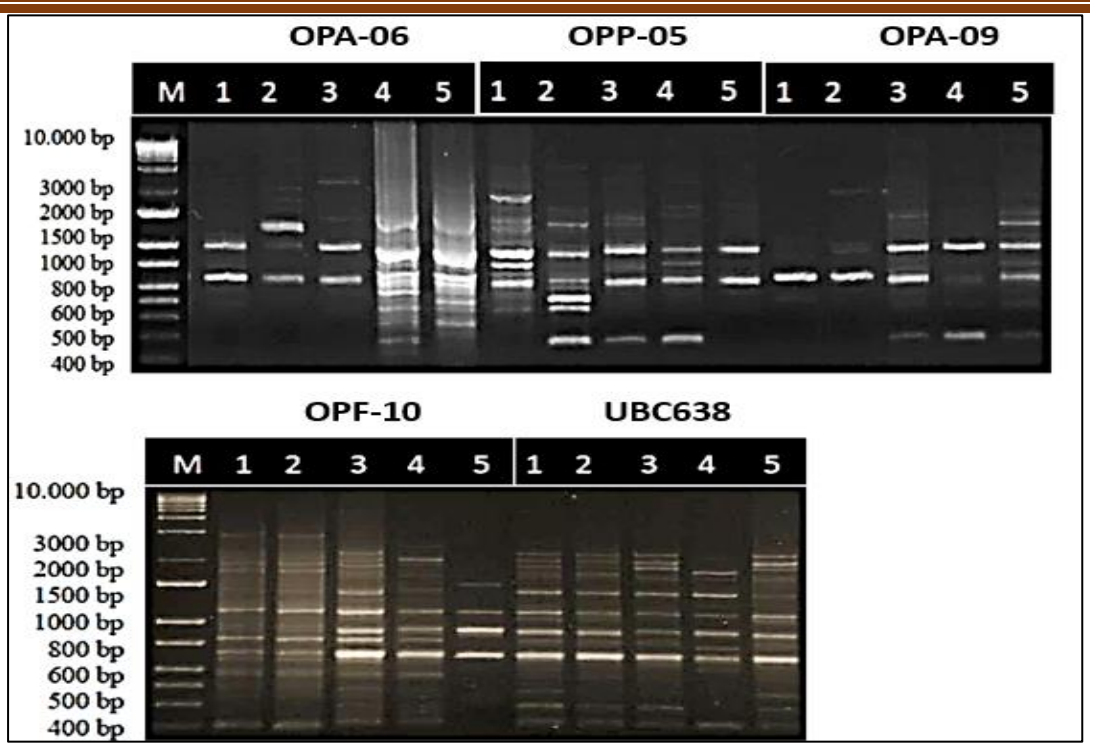

Fig. 1: RAPD profile of the studied wheat genotypes (marked as 1 to 5 for G1 to G5 respectively) using primers OPA-06, OPP-05, OPA-09, OPF-10 and UBC638M= O'GeneRuler DNA Ladder 0.1 µg µL^{-1}

Genetic similarity: The genetic similarity of the genotypes used in this study (Table 5) was determined according to (11). Cluster analysis (Figure 2) was applied using UPGMA method to group genotypes according to their similarity. Figure 2 shows the genotypes positions in different clusters. The generated dendrogram demonstrated that the studied genotypes fell into two main clusters, cluster A included the genotype G5 only, while the other four genotypes were grouped together in cluster B which divided into two subgroups. Genotypes G1 and G3 showed similarity being in same sub-subgroup B2b. RAPD appeared to be a reliable method for determining similarity between genotypes and this was proved by previous studies also (3, 6 and 12). Although it was only 5 genotypes were used in this study, however, genotypes showed good diversity to be used in breeding programs.

Table 5: Genetic similarity matrix of the five wheat genotypes based on RAPD data.

Genotypes	G1	G2	G3	G4	G5
G1	1.00				
G2	0.935	1.00			
G3	0.972	0.943	1.00		
G4	0.912	0.925	0.933	1.00	
G5	0.855	0.871	0.442	0.871	**1.00**

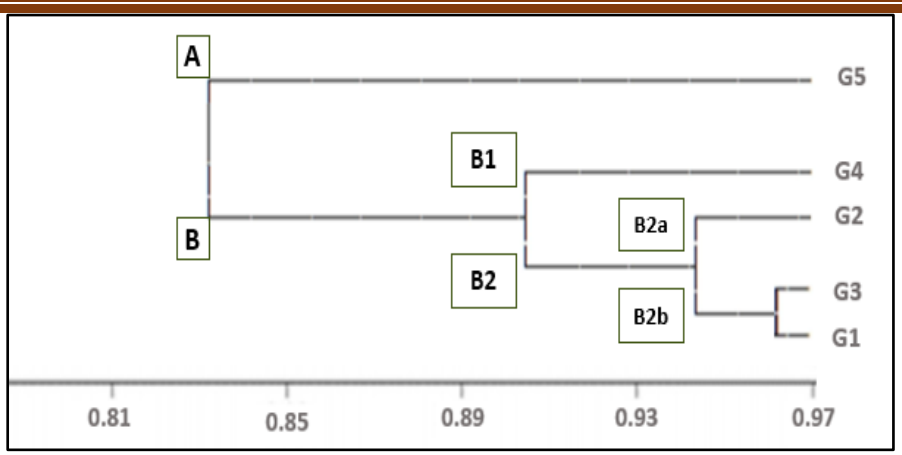

Fig. 2: Dendrogram of the genetic similarity among the studied wheat genotypes based on RAPD data.

Fingerprint: Results in Table 6 showed that primers OPA-06, OPP-05 and OPF-10 produced the highest number of the unique bands at 3, while the lowest was 1 band by OPA-09. Primers producing a unique band refers to those that are able to recognize a unique annealing site in plant genome which enhances the chances for producing a unique genotype-fingerprint, ; Such competence was mentioned by (3, 6 and 12).

Table 6: Number and size of the unique bands produced by RAPD primers and genotype-fingerprinting.

Primer	No. of unique bands	Band size (bp)	Genotype-fingerprint	No. of genotype-fingerprinting
OPA-06	3	3000, 2000, 1500	2	1
OPP-05	3	2000, 600, 800	1, 2	2
OPF-10	3	4000, 2000-1500	1, 2, 3	3
UBC638	2	1500, 800	4, 5	2
OPA-09	1	800	1, 2	2

Primer OPA-06 produced a distinctive 3000bp-sized band in genotype 3 and distinctive band in genotype 2 with molecular size of 2000 bp. In addition to other bands with molecular size appeared in both genotype 4 and 5 which didn't appear in other ones. Primer OPP-05 produced a unique reproducible band for the genotype G2 sized 2000 bp, in addition to two unique bands in both G4 and G5 with size of 600 bp and 500bp. Genome of genotype G1 and G2 showed uniqueness by giving special bands for both when using primer OPA-09. Primer OPP-05 showed one unique band for the genotype G1 with size of 3000pb, and two unique bands for G2 (800 and 600 bp). Primer UBC638 acted similarly in all genotypes by producing 8 bands for all except G4 (6 bands only) and G5 appeared with one band missing at molecular size of 1500bp.

Conclusions

RAPD analysis is widely used to determine divergence between genotypes of different crops and used extensively in wheat to characterize varieties. This study showed that RAPD approach appeared to be a reliable tool to identify and discriminate variation between wheat genotypes. As such, the unique bands produced from RAPD markers can be further improved as a sequence characterized amplified region (SCAR)

marker for simple and rapid identification of new genotypes of wheat in breeding. We believe that the primer OPF-10 could be the best primer to use in marker-assisted selection programs to investigate the polymorphisms of wheat genotypes. The studied genotypes, especially G1, G3 and G5, showed reliable diversity making them promising genetic materials in the selection and hybridization of wheat breeding programs in Iraq.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Author 1: conducting the experiment, data collection, and original draft writing; Author 2: data analysis and reviewing. Both authors have read and agreed to the published version of the manuscript.

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The study was conducted following the protocol authorized by the Head of the Ethics Committee, University of Anbar, Republic of Iraq.

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No Data Availability Statement.

Conflicts of Interest:

The authors declare no conflict of interest.

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