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DETERMINATION OF GENETIC VARIABILITY BETWEEN TRADITIONAL IRAQI WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES USING RAPD –MARKER ANALYSIS

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Abstract

Genetic variability evaluation among different species is an significant tool for genetic improvement to desirable quality and maximize the yield which ultimately increasing traditional plant breeding methods. Most efficient way of diversity evaluation is application of molecular markers. This study, twelve (RAPD) Random Amplified Polymorphic DNA Operon primers were applied to estimate the genetic variability between ten wheat (*Triticum aestivum* L.) genotypes. RAPD markers were performed to fingerprint ten genotypes. Overall 958 bands were amplified by 12 primers. RAPD data showed 90 polymorphic bands across tested genotypes. Each selected primer produced between 56 bands (OPA-11) to 110 bands (OPC-10). Largest number of polymorphic bands were 17 bands produced by primer OPC-10 while, Lowest number of polymorphic were 3 bands produced by OPA-11 and OPC-04 primers. The primer efficiency ranged from 0.15 in primer OPC-10 to 0.04 in primer OPC-04. The highest value of polymorphism and discrimination in this study was obtained from primers OPC-10, while, the lowest value of discrimination was obtained from primers OPC-04. The lowest genetic distance was 0.4103 between genotypes Rasheed and Iraq, while highest genetic distance was 0.8932 between genotypes Furat and Iba'a 99. Cluster analysis or dendrogram based on RAPD results displayed two major distinct groups 1 and 2. In this study reveals novel information about Wheat genome which can be used in future studies for wheat improvement and breeding program.

Key words : RAPD, Wheat, Polymorphism, Genetic diversity.

Introduction

Wheat **(***Triticum aestivum* L.) is one of the generality economically significant annual cereal crops and consider first strategic field crop for most of the world's population. It provides the world with 55% of total carbohydrates, 20% of food calories consumed, 12-17% of protein. Wheat forms the basis of food for almost 50% of the world population, because it contains the protein clotenin by 30-35%, which is the basis for the production of high-quality bread (FAO, 2011).

The great size of the genome and a rise reach of utilize have given wheat an significant agricultural and nutritional state among many different grain crops. A growing population and lifestyle changes have posed defiance for wheat farmers to increase state-of-the-art high-yielding wheat varieties with high-quality seeds, resistant to pests and stress conditions (Abd-El-Haleem *et al.,* 2009). Therefore, over the past few decades the breeding of wheat particles has acquired importance. Technology of DNA tag is an significant area of biotechnology that can certainly improve the efficiency of plant breeding exercises (Basel, 2012).

The estimation of genetic relationships between the members would raise the effective utilize of genetic variance in the breeding programme (Saengprajak and Saensouk, 2012).

Molecular biology present several techniques that can be used for plant identification. Genetic polymorphism in plants has been most studied which helps in distinguishing plants at inter- and/or intra-species level (Joshi *et al.,* 2004). RAPD based on PCR methods can be effectively used for the study of genetic diversity and phylogeny (Williams *et al.,* 1990). RAPD markers have been applied

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for the identity of genotypes and for estimation genetic diversity between of several crops genotypes such as wheat (Ahmad, 2014).

El-Sanousy *et al.,* (2015) determined genetic diversity among 75 wheat genotypes (66 hexaploids and 9 tetraploids) was studied using 6 RAPD primers. While, from another study, 12 genotypes of wheat generated during hybridization programme were examined for genetic diversity by RAPD marker (Bibi *et al.,* 2009).

Identity of plant genotypes is fundamental not only to effect their phylogenetic relationships but also to provide beneficial information for agricultural trading (Asif *et al.,* 2005).

In this study was perfected as a part of wheat breeding programme to estimate the genetic diversity between wheat genotypes using RAPD technique.

Materials and Methods

Samples Collection

Wheat genotype seeds were selected from different origin and certified sources in the country, such as: (1) Rasheed, (2) Fateh, (3) Tamose 2, (4) Nakhoa, (5) Furat, (6) Abu Ghraib 3, (7) Iba'a 99, (8) Dijlah, (9) Iraq and (10) Tahadi. Its seeds were sown during the growing season of 2018/2019 at Administration of Agriculture in Najaf, Coated Agriculture project. Seedling at age of 2 weeks becomes ready to take apical fresh leaves for genomic isolation of DNA.

DNA Extraction

Total genomic was extracted of DNA from each collected sample from leaves using standard Genomic DNA Mini Kit (Geneaid Biotech. Ltd; Taiwan Company) protocol with slight modification (Sambrook and Russell, 2001).

Confirm the DNA extraction, samples were sprint on 1.2 % agarose gel. DNA quantification was measured by using Nanodrop ND-1000 (Thermo Scientific). DNA dilutions were prepared for PCR reaction.

RAPD Analysis and Electrophoresis

12 RAPD primers were optimized and used for DNA amplification of Wheat germplasm and a sequence was amplified individually using oligonucleotide primer (Table 1), through (polymerase chain reaction) PCR. According to the Experimental Protocol ofAccuPower® TLA PCR PreMix. RAPDs were applied to determine the genetic variability among wheat genotypes.

Amplification was performed in thermocycler programmed according to annealing temperatures as follows:

Table 1: List of primers used in the study with sequences.

Primer	Sequence (5'→3')	Primer	Sequence (5'→3')
OPA-05	AGGGGTCTTG	OPC-04	CCGCATCTAC
OPA-11	CAATCGCCGT	OPC-10	TGTCTGGGTG
OPA-13	CAGCACCCAC	OPD-08	GTGTGCCCCA
OPA-18	AGGTGACCGT	OPF-05	CCGAATTCCC
OPA-19	CAAACGTCGG	OPF-14	TGCTGCAGGT
OPC-02	GTGAGGCGTC	OPJ-06	TCGTTCCGCA

Initial denaturation	**Temp.: 95°C**	**Time: 10 min**
No. of cycles = 40 cycles		
Denaturation	Temp.: 94°C	Time: 1 min
Annealing	Temp.: 37°C	Time: 1 min
Extension	Temp.: 72°C	Time: 1.2 min
Final extension	**Temp.: 72°C**	**Time: 5 min**

Then amplified DNA was isolated by electrophoresis in 1.2 % agarose gels then stained with ethidium bromide for (3 hr, 70V). Products of PCR were visualized by U.V. transilluminator and then were imaged through gel documentation system (Al–Badeiry *et al.,* 2013), the size of RAPD-PCR products evaluated by comparing with 1Kb DNA ladder marker (250-10,000) bp.

Scoring Data of RAPD Products

Bands scoring were done by using (0) for absent and (1) for present of bands in order for acquire the genetic similarity matrix. The genetic similarity was analyzed through program of NTSYS-PC (Numerical Taxonomy and multivariate Analysis System), software version 1.8 (Applied Biostatistics) program (Rohlf, 1993) to measure genetics distance among wheat genotypes.

Amplified bands, polymorphic bands number and (PIC) percentage of polymorphism information content was calculated by counting the reliable visible bands. This data were analyzed using SIMQUAL (Similarity for Qualitative Data) routine to create genetic similarity index (Nei and Li, 1979).

Dendrogram was created (UPGMA) Unweighted Pair-Group Method with Arithmetic average using the Dice's and Jacquard's coefficient of similarity to produce the most logical results (Rohlf, 1993), for molecular relationship between ten genotypes.

Primer efficiency, Polymorphism and discriminatory value were calculated for each primer using three equations as described by Graham and Mc Nicol (1995). In addition, DNA fingerprints were calculated by determining the number of the amplified bands (unique pattern for RAPD-PCR products) and their molecular sizes for each wheat genotype using the specific primers

in the present study.

Results and Discussion

Level of Polymorphism

The isolated DNA (concentration ranges from 200 to 250 ng/il) with purity of 1.8%1.9 specified by spectrophotometric ratio A260/A280. List of twelve RAPD primers were tested with ten individual DNA samples that amplified 958 bands

These data were important to reflect the usefulness of RAPD-PCR markers in analyzing wheat genome at molecular level.

Effective Polymorphic Information

For individual RAPD marker , The number of amplified bands, number of polymorphic bands, percentage of polymorphism information contents (PIC), primer efficiency was noticed.

Using several operon primers the highest number of main and amplified bands obtained across all genomes tested was generated by primer OPC-10, OPA-05 and OPA-18 respectively (Figure 1), the less number of main and amplified bands obtained was generated by primer OPA-11 as observed in (Table 2).

It is important to know that some primers recognize a high number of annealing site, which is more useful than primers recognizing lower number of annealing sites. In this case the number of amplified bands will be higher, thus giving a better chance for detecting DNA polymorphisms among individuals (Williams *et al.,* 1990).

Among all primers tested in this study, it was found that primer OPC-10 had the highest calculated efficiency which was 0.15, while, the primer OPC-04 had the lowest calculated efficiency which was 0.04 as shown in Table 2. The range of primer efficiency may show the ability of primer to give a large proportion for polymorphic bands according to total number of amplifying bands. The primer efficiency may differ from one template DNA of an organism to another (Newton and Graham, 1997).

Discrimination power of a primer will increase by increasing the number of identified varieties using the selected primers (Arif *et al.,* 2010). The highest polymorphism and estimate of discrimination in this study was obtained from primers OPC-10 which gave the elevated numbers of polymorphic bands, while, the lowest polymorphism and value of discrimination was obtained from primers OPC-04 which gave the lowest number of polymorphic bands as illustrated in (Table 2).

The possible reason is that primer could show enough polymorphism if the genetic differences existed (Patil *et al.,* 2010), or concern with variability in morphological traits that could produce different RAPD patterns (Abd El-Hady *et al.,* 2010).

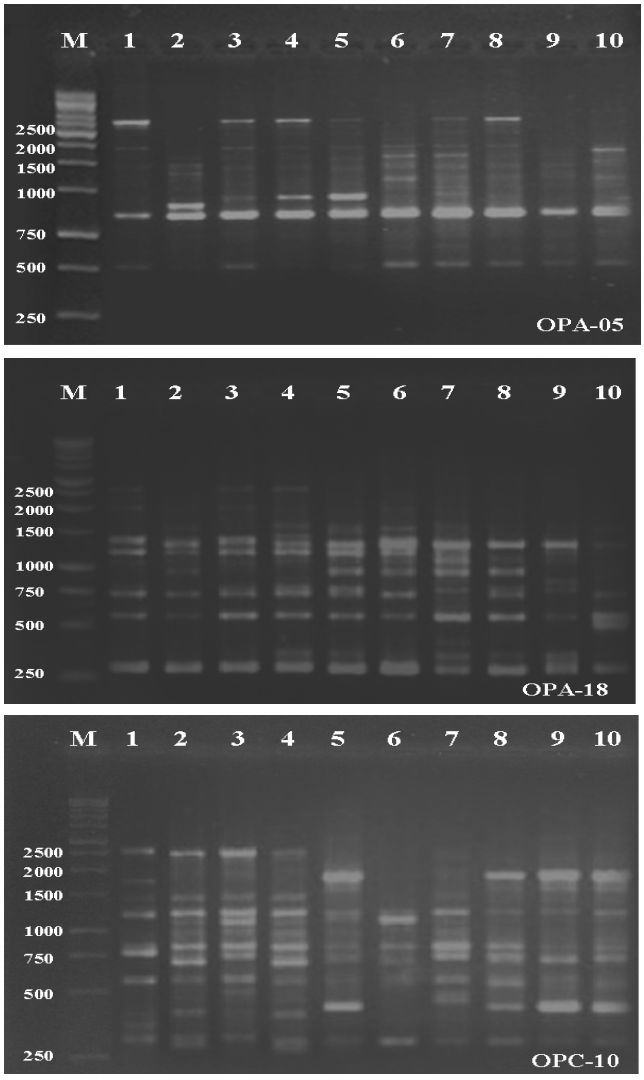

Fig. 1: Profiles of RAPD marker from the genomic DNA *T. aestivum* produced by OPA-05, OPA-18 and OPC-10 primers using 1.2% agarose gel, M: DNA ladder , Lanes: 1-10 which wheat genotypes.

Results of discriminatory power can be explained by the ability of primers to reflect the variation in individual genome according to the total number of variations, therefore, it is noted that the primer which has high ability of recognition is that primer which has the ability to give the highest polymorphic bands number according to the total number of variations (International Union for the Protection of New Varieties of Plants, 2010).

Power discrimination was also used to reveal the fingerprinting of other plants genomes such as Potato (Al-Hassani, 2002), Barley (Al-Asie and Jubrael, 2000) and Rice (Al-Musawi *et al.,* 2019). Discrimination power reflects genetics and is very important for plant breeders

Table 2: Summarized of No. of main bands, amplified bands, polymorphic bands, polymorphism, primer efficiency and discrimination value of each RAPD primer.

No.	Primer	No. of main bands	No. of amplified bands	No. of polymorphic bands	Polym orphism (%)	Primer efficiency	Discriminatory value (%)
1	OPA-05	16	99	13	81	0.13	14.4
2	OPA-11	6	56	3	50	0.05	3.3
3	OPA-13	9	61	7	78	0.11	7.8
4	OPA-18	15	93	12	80	0.13	13.3
5	OPA-19	10	70	7	70	0.10	7.8
6	OPC-02	11	85	5	45	0.06	5.6
7	OPC-04	8	77	3	38	0.04	3.3
8	OPC-10	20	110	17	85	0.15	18.9
9	OPD-08	10	65	5	50	0.08	5.6
10	OPF-05	13	84	8	62	0.10	8.9
11	OPF-14	10	81	6	60	0.07	6.7
12	OPJ-06	10	77	4	40	0.05	4.4
Total		138	958	90			

to see large variation for selection in future studies.

Genetic Similarity Matrix and Cluster Analysis

Results in table 3 present calculate the genetic distances among ten wheat genotypes. Dendrogram were created through UPGMA. Genetic similarity between ten genotypes was varying from the less genetic distance was (0.4103) between genotypes Rasheed and Iraq which that is the presence of similarity between two genotypes is high degree using RAPD markers bounded with morphological characters between these two genotypes. On the basis of morphological features and traits, it was found that these two genotypes have some similar traits, like their number of leaves per plant, plant height and leaf area per plant (Wattoo *et al.,* 2009), also which indicates that these genotypes are closely related to each other (Table 3).

Highest genetic distance was (0.8932) between genotypes Furat and Iba'a 99 which refers that the presence of similarity among them are very low and they were introduced from different geographical origins.

Presence of some common morphological characters between genotypes will help to increase the present of genetic similarity between studied genotypes using RAPD marker and this agrees with (Bai *et al.,* 1997) who found that the degree of genetic similarity between medical plant variety (ginseng) by applying RAPD marker is high when he selected long plants only compared with genetic similarity between random samples of the same species.

Wild populations of *Mentha aquatica* in Iran show similarity ranged (0.21-0.79) screened from twenty markers were selected for RAPD analysis. Determination of difference among species particular that RAPD marker is convenient approaches to investigates the polymorphic loci and to evaluate genetic distance among the populations of the species (Kazemi and Hajizadeh, 2012). Choumane *et al.,* (2004) evaluated genetic diversity by DNA markers (e.g. RAPD markers) between and within 21 populations of *Pinus brutia* collected from five different regions and from sites geographically close to each others.

The phylogenetic tree was another important issue to be determined on the basis of RAPD profiles obtained in this study. Aiming of generating a dendrogram is to

Table 3: Genetic distance values for wheat genotypes studied in RAPD analysis. The genotype numbers from 1 to 10 (see Materials and Methods)

Genotype	1	2	3	4	5	6	7	8	9	10
1	0.0000									
2	0.4861	0.0000								
3	0.6251	0.7645	0.0000							
4	0.5257	0.5883	0.8346	0.0000						
5	0.6330	0.7593	0.7707	0.7138	0.0000					
6	0.5847	0.6905	0.7224	0.6350	0.7241	0.0000				
7	0.6719	0.4962	0.7571	0.5506	0.8932	0.7005	0.0000			
8	0.7141	0.7404	0.7098	0.7616	0.5903	0.7162	0.7116	0.0000		
9	0.4103	0.4965	0.6481	0.5773	0.7619	0.5438	0.6431	0.7305	0.0000	
10	0.6644	0.7806	0.4719	0.7062	0.6304	0.7241	0.7853	0..7593	0.6453	0.0000

imagine the best representation of the overall similarity or phylogenetic (phylogeny) relationships among a group genotypes, individuals, cultivars, populations, or species.

According to this dendrogram it is possible to distinguish two main genetic groups (major clusters) Fig. (2), group 1 and group 2. The major group A comprised 4 genotypes, while the cluster B comprised six genotypes. Cluster A which further divided in in to two equal subclusters, the first included, **(**5) Furat and (8) Dijlah while second subclusters included, (3) Tamose and (10) Tahadi genotypes. Cluster B Consist of six genotypes which further divided in to subclusters, the first major included (5) genotypes (4) Nakhoa, (7) Iba'a 99, **(**9) Iraq, (2) Fateh and **(**1) Rasheed, while the second small included one genotypes : **(**6) Abu Ghraib 3, which indicates that it is the most diverse genotype among all other genotypes.

In this study are in agreement with the results of Onto *et al.,* (2008) studies involving genus *Cucumbers*

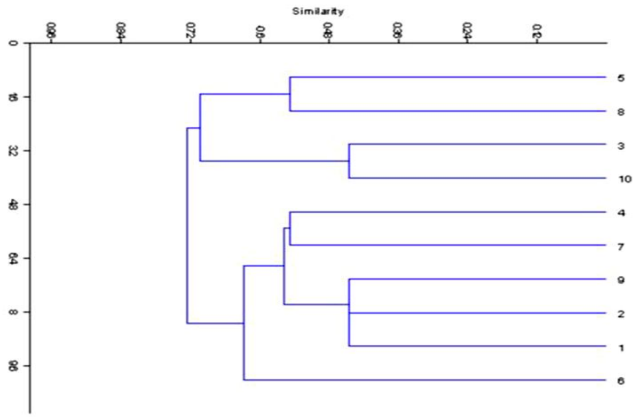

Fig. 2: UPGMA dendrogram illustrating the tree of genetic relationship between wheat genotypes using RAPD markers.

Lanes: (1) Rasheed, (2) Fateh, (3) Tamose 2, (4) Nakhoa, (5) Furat, (6) Abu Ghraib 3, (7) Iba'a 99, (8) Dijlah, (9) Iraq and (10) Tahadi.

studied could be separate into two major subgroups in that the similarity values were between 0.67-0.93. And highlights the importance of finding the genetic distance between genotypes studied to help plant breeders in making the right decision by choosing appropriate parents to form new genetic consensus, especially when developing plants in terms of increasing resistance to pathogens and unfavorable environmental conditions (Weeden *et al.,* 1992).

Conclusion

Aim of present research were to evaluate the genetic information using RAPD markers and data generated by these primers displays the effectiveness of primers and provides primer system for genetic analysis. Molecular markers are widely used to define the genetic variations, as molecular markers are self-defining for confusing results of environment factors. Genetic variability is the key determinant for any breeding program. This study detects that PCR based fingerprinting technique, RAPD were informational for assessing the range genetic diversity as well as to separate the genetic relationship between various species of *T. aestivum.* Outcomes of this study committed that RAPD primers have the capacity to measure, similarities, polymorphism and identification of genotypes using specific RAPD markers for wheat genotypes

In future our research work is perhaps helpful for inexpensive and better RAPD analysis of new genetic species diversity. The genetic relationships among these genotypes should be useful in choosing of genetically special parents for germplasm development. Finding of comparatively trivial genetic changing in wheat confirm the requirement to expand the genetic base of wheat breeding materials. Estimation of genetic diversity may also be beneficial in choosing divergent parents for genome mapping objectives. All these, research provides important knowledge for breeder to develop genotype resource and approve mentioned primers to be applied in wheat breeding programs as good tools in assessing genotype classification and useful for wheat improvement programme.

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