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GENETIC RELATIONSHIP AMONG TEN WHEAT GENOTYPES USING SEVENTEEN RAPD MARKERS

Melath Kadem Farhood AL-Ghufaili and Attyaf Jameel Thamir Al-Tamimi*

Faculty of Science, University of Kufa, Iraq.

Abstract

This study was conducted for determination DNA fingerprint and estimation of genetic diversity among ten wheat (*Triticum aestivum* L.) genotypes using seventeen RAPD Markers. Primers OPB-06, OPC-05, OPH-01 and UBC-126 gave unique fingerprint for studied genotypes. Primer OPG-09 gave higher value for polymorphism . The higher efficiency and discriminatory value was produced by primer UBC-126. High genetic distance was 0.546 between Buhuth22 and Faris while low genetic distance was 0.142 between Rasheed and Iraq. Cluster analysis (Phylogenetic tree) by UPGMA based dendrogram revealed that studied genotypes grouped in two main clusters. Low polmorphisim (36.827) revealed by studied primers. Results show that RAPD markers could efficiently reveal genetic variation and fingerprint wheat genotypes .

Key words : Wheat, RAPD markers, genetic distance ,cluster analysis , polymorphism.

Introduction

Characterization of genotypes is conducted by different markers including, morphological (Wettstein-Knowles, 1992), biochemical (Kumar *et al*., 2009) and DNA markers, which defined as a sequence of DNA or a gene situated on chromosome (Collard *et al*., 2005 and Schulmann, 2007) by which it could detect differences between individuals through showing polymorphism (Collard *et al.,* 2005). The major step in crop improvement including wheat is the complete molecular characterization of its germplasm , genetic relationship and genetic diversity among breeding lines could help in strategies used for crop improvement (Abbas *et al*., 2008). Genetic diversity of plants determines their potential for improvement and their use for breeding, which enhanced food production. (Khodadadi *et al.,* 2011).DNA markers are considered very efficient in selection of plant material for their independent of environment, their segregation as single genes and that DNA could extracted easily from plant materials. (Ovesna *et al.,* 2002),these markers could be useful in identification of genetic materials, selection of parents, detection of progeny and characterization of the varieties for protection both consumers and breeders rights. (Ovesna *et al*., 2002).

RAPDs (Random Amplified Polymorphic DNA) need small quantities of DNA and do not require radioactive labels, they are simple and fast and have proven to be an important tool in detecting genetic diversity and identification of plant species germplasm. (Solimana *et al*., 2014).

There is a great important of studying germplasm genetic composition of cultivars and comparing them with their related ancestors, this will provide information about their phylogenetic relationship and produce a chance to find new useful genes , so this study was conducted for studying genetic relationship and revealing genetic variation among wheat genotypes through using Random amplified polymorphic DNA (RAPDs) and determination of primers capable of offering unique fingerprint for wheat genotypes ,the obtained data will help in management of breeding programs through guiding breeder for choosing suitable parent in hybridization.

Materials and Methods

The study conducted using ten wheat genotypes (1- Furat 2- Baghdad 3- Hashimia 4- Buhuth22 5- Latifia 6- Dijla 7- Abaa 99 8- Rasheed 9- Faris 10- Iraq). Seedling

***Author for correspondence :** E-mail : atyaf.altameemi@uokufa.edu.iq

at age of (3-4) weeks becomes ready to take apical fresh leaves for genomic DNA extraction.The Genomic DNA Mini Kit (Geneaid Biotech. Ltd; Taiwan Company). Seventeen primer were provided by Bioneer Corporation in lyophilized form, dissolved in TE buffer to obtain 100 pmol/µl as a final concentration (stock solutions) ,their names and sequence shown in table 1.

PCR Pre Mix master mix by Bioneer Corporation USA contain (DNA polymerase(1unit), each: dNTP(250 µM), Tris-HCl (pH 9.0, 10 mM), KCl(30 mM), $MgCl_2$(1.5 mM) and stabilizer and tracking dye (5 µM). According to the Experimental Protocol of AccuPower® TLA PCR PreMix, the PCR reaction mixture was prepared by using 6µl template DNA and 3 µl of primer (10 pmole/µl), were added to each AccuPower® TLA PCR Pre Mix tube. Sterilized deionized distilled water was added to final volume of 20 µl. Reaction was performed in Thermo cycler Agilent technology surecycler 8800, programmed as mentioned by Naghavi *et al*. (2004), El-Assal and Gaber (2012) and Ezekiel *et al*. (2011). The gel electrophoresis methods were done according to Sambrook and Russel (2001). RAPDs amplified product were separated by electrophoresis on 1.2 % agarose gels (3-4 hrs at 70V). The photographs resulted from agarose gel electrophoresis was used to score data, presence of a product was identified as (1) and absence was identified as (0), data then entered into PAST statistic vital program, Version 62.1 (Hammer *et al*., 2001).

Results and Discussion

RAPD profile results showed variation among studied genotypes through presence of monomorphic, polymorphic and unique bands. Primers OPB-06, OPC-05, OPH-01 and UBC-126 gave unique fingerprint for each genotype (figs. 1-4), while primers OPA-14, OPA-17, OPC-09, OPF-20, UBC-116 and UBC-129 failed to give unique fingerprint ,other primers ranged (1-6) in their fingerprinted genotypes as in table 2. Primers, which gave a unique fingerprint are those who produced high number of unique band, this indicate that every genotype had one or more novel or specific sequences which was not found in other genotype ,these bands can be efficiently used as genetic markers for identification of these genotype to differentiate specific genotype from others (Grewal *et al*., 2007; Vishwanath *et al*., 2010; Fadoul *et al*., 2013 and AL-Tamimi, 2014).

Results in table 3 shows all RAPD data,in which higher molecular size was 3603 bp while the lower molecular size was 174bp, these variation related to primer sequence annealed with DNA template (Mahpara *et al*., 2012). The higher number of main and amplified bands was 21 and 116 respectively .Variation in number of main and amplified bands are mainly due to primer structure and 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 and Tahir, 2014). Presence of monomorphic bands (2-8 bands) refer to that genome contains constant identical sequences commonly refer to as conserved sequence (Al-Judy, 2004). monomorphic bands are type of these sequence, which reveal that genotypes belong to one species and share some genome sequences and differ in others (Russel *et al*., 1997; Al-Judy, 2004 and AL-Badeiry, 2013 and AL-Tamimi, 2014).

The polymorphic bands reached to 15 band , which increase chance of a better characterization of genotypes. (Demir *et al*., 2010). OPG-09 gave higher polymorphism value reached 82.35. Polymorphism always concerned with absence and presence of bands, its value increased with increasing number of polymorphic bands (Hunter and Gaston, 1988 and Graham and McNicol, 1995). Difference in level of polymorphism differ due to difference in number of germplasm ,their origin and number of primers used (Qadir *et al*., 2015). polymorphism among cultivars could arise through changes in nucleotide sequence, which prevent the amplification by a mismatch at primer binding sites through deletion or insertions, this could changes size of the amplified product (Powell *et al*., 1996 and Fadoul *et al*., 2013). Increasing of primer polymorphic bands result in that this marker could be used in further as polymorphic marker could successfully identify and examining genotypes purity of crops (Pal and Singh, 2013). The number of unique bands reached to six in primer OPH-01, the presence of such bands refer to that primer recognized a unique annealing site in genome ,this increase chance of producing a unique cultivar fingerprint (Grewal *et al*., 2007; Vishwanath *et al*., 2010; Fadoul *et al*., 2013 and AL-Tamimi, 2014). Primer UBC-126 gave higher value for both efficiency and discriminatory value Both efficiency and discriminatory of primer concerned with its ability to give unique fingerprint. (Newton and Graham, 1997; Arif *et al.,* 2010 ; AL-Badeiry, 2013 and AL-Tamimi, 2014). Previous studies on some same primers produced different results. Agreement and dis agreement with other researchers is certainly related to diverse germplasm used.

In table 4, the results showed that the highest genetic

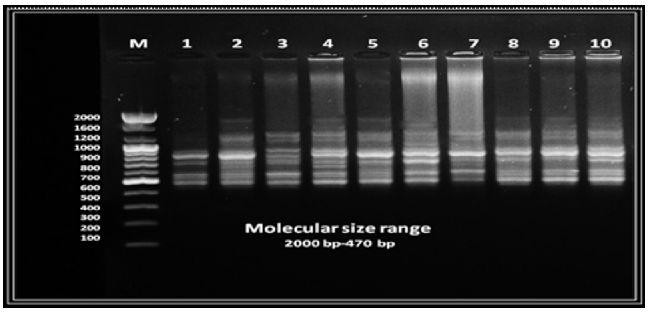

Fig. 1 : The amplification results obtained by primer OPB-06.

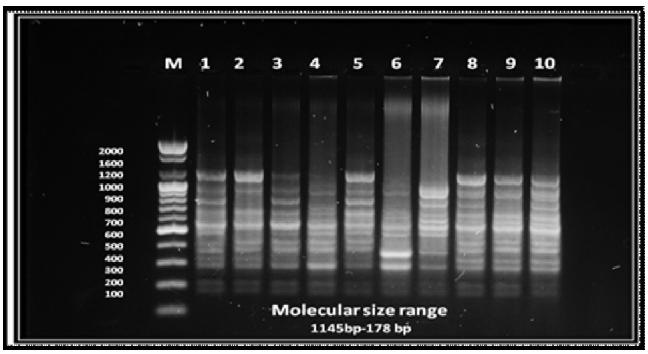

Fig. 2 : The amplification results obtained by primer OPC-.

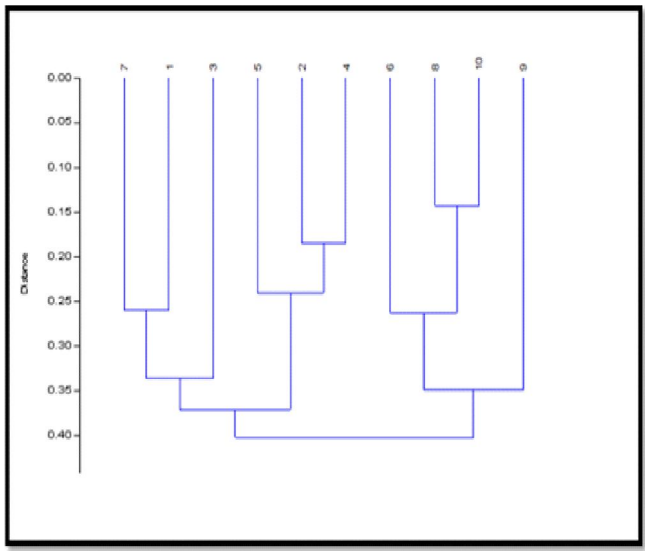

Fig. 5 : UPGMA dendrogram illustrating the trees of genetic relationship between wheat genotypes using RAPD markers Wheat genotypes: 1.Furat 2.Baghdad 3.Hashimia 4.Buhuth22 5.Latifia 6. Dijla 7.Abaa99 8. Rasheed 9. Faris 10. Iraq.

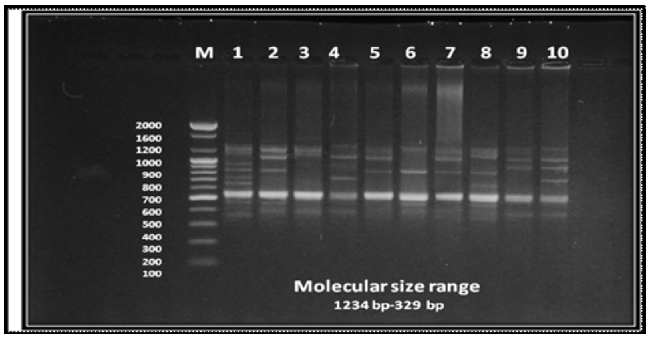

Fig. 3 : The amplification results obtained by primer OPH-01.

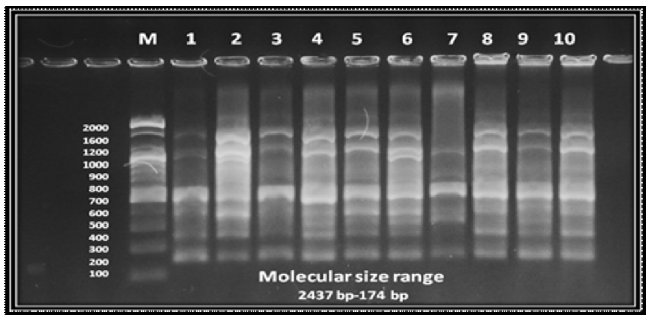

Fig. 4 : The amplification results obtained by primer UBC126.

distance was observed between Buhuth22 and Faris genotypes while lowest genetic distance was between Rasheed and Iraq genotypes. Genetic distance between genotypes and identification of parents is beneficial for performing suitable crossing to reach maximum heterosis through hybridization programs. RAPDs are useful in determination of phylogenetic relationships among cultivars. Cultivars with the most distinct DNA profiles were likely to contain the greatest number of novel genes. (Ashraf *et al*., 2003 and Fadoul *et al*., 2013). Different and similar morphological character may result in low and high genetic distance.(Vieira *et al.,* 2007), especially those character that their site on genome could be recognized by markers and result as presence of band, these bands which consider the base for building genetic distance among genotypes, especially novel genes,in general, when there were maximum distance among genotypes, this resulted ingiving high yield, crossing among these genotypes through breeding programs to minimum distance could be used in backcross breeding programs

Table 1 : Primers name and their sequences which have been used as RAPDs markers.

Primer	Sequence(5'-3')	Primer	Sequence(5'-3')	Primer	Sequence(5'-3')
UBC112	GCTTGTGAAC	UBC128	GCATATTCCG	OPF-20	GGTCTAGAGG
UBC114	TGACCGAGAC	UBC129	GCGGTATAGT	OPG-09	CTGACGTCAC
UBC116	TACGATGACG	OPB-06	TGCTCTGCCC	OPH-01	GGTCGGAGAA
UBC117	TTAGCGGTCT	OPA-17	GACCGCTTGT	OPA-14	TCTGTGCTGG
UBC126	‘CTTTCGTGCT	OPC-05	GATGACCGCC	OPA-15	TTCCGAACCC
OPC-08	TGGACCGGTG	OPC-09	CTCACCGTCC		

Table 2 : Wheat genotypes fingerprinting (DNA profile) using 17 RAPD primers.

Primer	Unique fingerprint	Primer	Unique fingerprint	Primer	Unique fingerprint
OPB-06	1-10	UBC-128	2,10	OPA-14	0
OPC-05	1-10	UBC-112	1	OPA-17	0
OPH-01	1-10	UBC-114	1	OPC-09	0
UBC-126	1-10	OPG-09	1,2,4,6,7,10	OPF-20	0
OPC-08	7,9	UBC-117	5,6,10	UBC-116	0
OPA-15	3,4,5	UBC-129	0		

Table 3 : Summarized results of RAPDs amplification product include :1-fragment size range in bp 2- No. of : main bands 3- No. of amplified bands 4- No. of monomorphic bands 5- No. of polymorphic bands 6-No. of unique bands 7-polymorphism(%) 8- primer efficiency and 9-discriminatory value(%).

Primers	1	2	3	4	5	6	7	8	9
OPA-14	268-1783	**9**	82	8	1	0	11.1	0.01	1.25
OPA-15	301-2000	12	72	6	2	4	16.6	0.02	2.5
OPA-17	534-1552	6	60	6	0	0	0	0	0
OPB-06	470-2000	16	93	3	10	3	62.5	0.1	12.5
OPC-05	178-1145	17	116	5	10	2	58.8	0.08	12.5
OPC-08	309-987	9	72	4	5	0	55.5	0.06	6.25
OPC-09	316-1667	11	93	8	3	0	27.2	0.03	3.72
OPF-20	250-1226	4	37	3	1	0	25	0.02	1.25
OPG-09	274-2000	17	89	2	14	1	82.3	0.15	17.5
OPH-01	329-1234	17	68	3	8	6	47	0.11	10
UBC-112	280-1328	8	64	6	1	1	12.5	0.01	1.25
UBC-114	643-2190	6	53	4	2	0	33.3	0.03	2.7
UBC-116	391-915	6	52	5	1	0	16.6	0.01	1.25
UBC-117	193-1776	11	77	6	3	2	27.2	0.03	3.75
UBC-126	174-2437	21	92	2	15	4	71.4	0.16	18.72
UBC-128	394-3603	7	43	3	2	2	28.5	0.04	2.5
UBC-129	640-2861	4	30	2	2	0	50	0,06	2.5

Table 4 : The genetic distance values among wheat genotypes using RAPD markers.

0	1	2	3	4	5	6	7	8	9	10
1	0									
2	0.45128	0								
3	0.33824	0.41497	0							
4	0.44283	0.18483	0.30854	0						
5	0.37351	0.23461	0.33727	0.24584	0					
6	0.43008	0.49702	0.46832	0.49843	0.44871	0				
7	0.25978	0.31697	0.33332	0.42618	0.26861	0.44869	0			
8	0.36579	0.36413	0.41774	0.41455	0.32563	0.25056	0.37853	0		
9	0.43935	0.37883	0.35401	0.54686	0.41873	0.32406	0.35402	0.37742	0	
10	0.29859	0.3585	0.35055	0.37952	0.33683	0.27577	0.37013	0.14282	0.34383	0

(Khodadi *et al*., 2011).

According to dendrogram produced in fig. 5 there were two main clusters, the first small cluster included genotypes Dijla, Rasheed, Faris and Iraq while the other large cluster included Furat, Baghdad, Hashimia, Buhuth22, Latifia and Abaa 99. Although, accessions with the same or adjacent geographic origin have the tendency to cluster together, accessions from different regions were also found to be closely related regardless of their geographic origin.

This suggests that selection of parent genotypes for breeding should not be based on geographical origin only because this is not always an accurate indicator of genetic diversity (Keneni *et al.,* 2005; Zvingila *et al*., 2005; Gashaw *et al.,* 2007; Celka *et al.,* 2010 and Sharifova *et al.,*2013).

Conclusion

The basic conclusion was that RAPD markers could be used in fingerprinting and revealing genetic diversity in wheat germplasm.

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