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GENETIC DIVERSITY AMONG BREAD WHEAT GENOTYPES USING RAPD AND SSR MARKERS

ATTYAF J.T. AL-TAMIMI[1]* and ALI S. AL-JANABI[2]

[1]Department of Biology, Faculty of Science, University of Kufa, Najaf, Iraq
[2]Department of Horticulture and Landscape Gardening, University of Kufa, Najaf, Iraq
*Corresponding author's email: atyaf.altameemi@uokufa.edu.iq
Email address of coauthor: ali.aljanabi@uokufa.edu.iq

SUMMARY

This study was accomplished during 2017-2018 with the aim to determine the genetic diversity among seven Iraqi bread wheat genotypes (Sham-6, AL-Noor, Tamuz-2, Tamuz-3, Abu-Ghraib, Baghdad and Dujlah) using PCR-DNA based nine RAPD and seven SSR markers at the Molecular Laboratory, Department of Biology, Faculty of Science, University of Kufa, Iraq. Among nine RAPDs primers, the primer OPC-09 showed the highest value for number of polymorphic bands, polymorphism, efficiency and discrimination. The highest numbers of amplified and monomorphic bands were produced by primer OPA-02. The highest number of main and unique bands was produced by primer OPB-07. Phylogenetic relationship constructed using RAPD markers grouped all the wheat genotypes in one large cluster except the genotype Dujlah. Higher genetic distance (0.3356) was observed between wheat genotypes Tamuz-2 and Dujlah while the lowest genetic distance (0.077) was recorded between wheat cultivars AL-Noor and Baghdad. Among seven SSR markers, analysis revealed that primer Xgw-m162 was more efficient by producing the highest values for allele number, gene diversity and polymorphic information content (PIC). Highest values for major allele frequency were produced by primers Wmc6037A, Xgwm608, and Xgwm6395B. Primers Wmc596, Xgwm1202B, and Xgwm2641B produced the highest values for heterozygosity. Highest value of PIC was produced by primer Xgw-m162 confirming its importance and found highly informative in studying the genetic diversity among the wheat genotypes. Dendrogram of phylogenetic relationship using SSR markers revealed that all the wheat genotypes were grouped in one large cluster except cultivar Tamuz-2. In future, such type of studies will serve as baseline for plant breeders to select appropriate parental genotypes to obtain highest desirable heterosis in wheat populations during hybridization.

Key words: Genetic diversity, RAPD and SSR markers, polymorphism, wheat genotypes, *Triticum aestivum* L.

Key findings: In the said molecular study, both RAPD and SSR markers revealed genetic variation even in this small group of wheat genotypes. Cluster analysis using both RAPD and SSR markers arrange the wheat genotypes independently irrespective of genetic relationship. However, overall the lowest genetic diversity was observed among studied Iraqi wheat genotypes and there is an intensive need to broaden the wheat genetic base.

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INTRODUCTION

Wheat belongs to family Graminae comprising a large number of important species in which 70% are used as human food (Pulhan *et al*., 2003; Pathak and Shrivastav, 2015). This is due to high protein and starch content which are about 60%-75% of total dry weight of the wheat grain (Belderok *et al*., 2000; Shewry, 2007). In addition, whole wheat grain has the ability of protecting against heart disease, and reducing the risk of hormone-related cancers like breast cancer (Kumar *et al*., 2011). Increased plant genetic diversity enhances its ability of improvement and as a consequence its use for breeding will eventually increase food security (Khodadadi *et al*., 2011).

Morphological characterization of crops requires evaluation through field trials which is time consuming (Astarini *et al*., 2004). Biochemical methods using SDS-PAGE (sodium dodecyl sulfate polyacrylamide agarose gel electrophoresis) is used as alternative method to morphological characterization for their simplicity (Sinha *et al*., 2012). These studies are mainly dependent upon seed protein or isoenzymes functions (Spooner *et al*., 2005). Studying wheat germplasm genetic variation depending upon morphological and biochemical markers as established in earlier studies (Attia *et al*., 2015; Al-Tamimi and Al-Rufaye, 2018). Genome complexity and size and differences in polyploidy of wheat made slow the development of molecular genetics as compared to other crops i.e., rice and maize, resulting low level of polymorphism and high percentage of repetitive sequences (Gupta *et al*., 1999).

For genetic variation, the molecular markers (DNA based markers) are used as tools to represent the different segments of DNA whose sequence is related to genomes which is responsible for a specific character (Bagali *et al*., 2010). Wheat possesses large size of genome and a wide range of uses with agronomic and nutritional values and thus, it an important crop among other cereal crops (Motawei *et al*., 2007).

Random Amplified Polymorphic DNA (RAPD) depends upon amplifying a random segment of DNA template using identical pair of primers about 8 to 10 bp in length with low annealing temperatures usually (36 to 40 °C) resulting a number of amplifying products representing different loci

(Liu and Cordes, 2004). Polymorphism resulted from different mutations and causes changing in primer annealing site (Welsh and McClelland, 1990). The main advantage of this technique is that it is very simple and cheap procedure, and the prior acknowledgement of template sequence is also not required (Weising *et al*., 2005; Edwards and Mccouch, 2007). In wheat, RAPDs are used to study many aspects including genetic relationships among cultivars (Al-Ghufaili and Al-Tamimi, 2018), genetic diversity and fingerprinting (Fadoul *et al*., 2013), identification of drought tolerant genotypes (Deshmukh *et al*., 2012) and detection of genotypes resistance to different pathogens (Patil and Hanchinal, 2011).

Simple Sequence Repeats (SSRs) are short nucleotides randomly repeated of motif (1-6) bases, and as a marker easily amplified, occurrence and distributed in even manner along genomes (Weising *et al*., 2005). This increases their ability to be polymorphic and specific (Bornet and Branchard, 2001). Their polymorphisms are related to unique locus. Variation in their length depends upon a number of repeats of a specific motif which in turn associated with DNA strands slip paging when DNA replicates (Schlotterer and Tautz, 1992). Many studies concerned with using SSRs on wheat genome including, genetic diversity (Kumar *et al*., 2009; Salehi *et al*., 2018), genotypes characterization (Islam *et al*., 2012), DNA fingerprinting (Kumar *et al.*, 2016) and resistant genes characterization (Ali *et al*., 2018). Therefore, the said study was conducted with the aim to determine the genetic diversity in seven Iraqi wheat genotypes through nine RAPD and seven SSR DNA markers, and to formulate the basic information about the existing wheat germplasm for building a future strategy for improvement in wheat crop.

MATERIALS AND METHODS

Plant material

Seven Iraqi wheat genotypes with diverse origin i.e., Sham-6 (Syria), AL-Noor (Mexico hybrid), Tamuz-2 (Mexico hybrid), Tamuz-3 (Mexico), Abu-Ghraib (Mexico), Baghdad (Australian strain × Aras), and Dujlah (American genetic structures 655 in their F_2 generation) were used to study the genetic diversity during 2017-2018 at the Molecular Laboratory, Department of Biology, Faculty of Science, University of Kufa, Iraq.

DNA markers

Nine RAPD Operon and seven SSR markers were used to study the polymorphism and fingerprinting for genetic diversity in different wheat genotypes, and the markers are illustrated with their sequence in Table 1.

DNA extraction

DNA was extracted from fresh apical leaves at age of three weeks using DNA Mini Kit, Taiwan. The isolated DNA concentration and purity were evaluated using Bio-drop apparatus Agarose gel electrophoresis for total genomic DNA accomplished using 1% agarose at 90 volt for about one hour following the procedure of Sambrook and Russell (2001).

Table 1. RAPD and SSR primers used for molecular analysis in seven Iraqi bread wheat genotypes.

No.	RAPD Primers		SSR Primers	
	Sequence (5'-3')	Primers	Sequaence (5'-3')	Primers
1	TTCGAGCCAG	OPC-01	F. CTC TCT CCA TTC GGT TTT CC R. CAT GCC CCC CTT TTC TG	Xgwm6395B
2	TGGACCGGTG	OPC-08	F-TCAGCAACAAACATGCTCGG R-CCCGTGTAGGCGGTAGCTCTT	Wmc596
3	CTCACCGTCC	OPC-09	F-ACAAACGGTGACAATGCAAGGA R-CGCCTCTCTCGTAAGCCTCAAC	Wmc6037A
4	CCGAATTCCC	OPF-05	F. GATCCACCTTCCTCTCTCTC R. GATTATACTGGTGCCGAAAC	Xgwm1202B
5	TGCCGAGCTG	OPA-02	F.GAGAAACATGCCGAACAACA R. GCATGCATGAGAATAGGAACTG	Xgwm2641B
6	GTGATCGCAG	OPA-10	F.AGT GGA TCG ACA AGG CTC TG R.AGA AGA AGC AAA GCC TTC CC	Xgwm1623A
7	TCTGTGCTGG	OPA-14	F. ACA TTG TGT GTG CGG CC R. GAT CCC TCT CCG CTA GAA GC	Xgwm6084D
8	TTCCGAACCC	OPA-15	-	-
9	GGTGACGCAG	OPB-07	-	-

PCR amplification

Both RAPD and SSR amplification were performed in 20 µl volume of amplification reaction of Maxime PCR Pre Mix Kit (i-Taq) including 7 µl of template DNA, 5 µl of reaction mixture containing (i-Taq DNA polymerase (5U/µl):2.5U, dNTPs 2.5 mM each, reaction buffer (10x):1x, gel loading buffer:1x), 5 µl of primer and final reaction volume of 20 µl completed by deionized distilled water and amplification were performed in a programmed Thermo Cycler, Agilent Technology Sure Cycler 8800/Malaysia).

RAPD markers amplification

For primers (OPB-07, OPC-01, and OPF-05), the amplification programmed as reported in past studies including (96 °C for 5 min as initial denaturation, 45 cycles of 94 °C for 1 min denaturation, 35 °C for 1 min annealing, and 72 °C for 2 min extension and final extension at 72 °C for 7 min (Al-Badeiry, 2013). For primers (OPA-02 and OPA-10), the amplification programmed including (94 °C for 5 minas initial denaturation, 40 cycles of 94 °C for 1 min denaturation, 40 °C for 2 min annealing, and 72 °C for 2 min extension and final extension at 72 °C for 5 min (Carelli *et al*., 2006; Abd-El-Hady *et al*., 2010). However, for primers (OPA-14, OPA-15, OPC-08, and OPC-09), amplification performed as including (94 °C for 3 minas initial denaturation, 45 cycles of 94 °C for 20 sec denaturation, 37 °C for 20 sec annealing and 72 °C for 40 sec extension and final extension at 72 °C for 10 min (Ezekiel *et al*., 2011).

RAPD markers amplification product electrophoresis

Product electrophoresis accomplished at 80 V using 1.2 % agarose for about two hours.

SSR markers amplification

Amplification programmed for primers Wmc596 and Wmc603 including (3

min as initial denaturation at 94 °C, followed by 35 cycles, each consisting of: 1 min at 94 °C, 1 min at 61 °C, 2min at 72 °C and a final extension of 10 min at 72 °C) (El-Assal and Gaber, 2012). However, for primers (Xgwm1202B, Xgwm264-1B, Xgwm1623A, Xgwm6084D and Xgwm6395B) amplification performed as (3 min as initial denaturation at 94 °C, followed by 35 cycles ,each consisting of: 1 min at 94 °C, 1 min at 55 °C, 2 min at 72 °C and a final extension of 10 min at 72 °C).

SSR markers amplification product electrophoresis

The electrophoresis accomplished for all the SSRs at 90 V using 2% agarose for about an hour and half.

Statistical analysis

RAPD analysis

Photographs resulted from agarose gel electrophoresis were used to score the data, presence of a product was identified as (1) while absence was identified as (0), data then entered into PAST statistic vital program, Version 62.1 (Hammer *et al*., 2001). Data were analyzed using SIMQUAL (Similarity for Qualitative Data) routine to evaluate genetic similarity index (GS) (Nei and Li, 1979). However, the dendrogram was constructed based on genetic distance (GD) using the Un-weighted Pair-Group Method with Arithmetical Average (UPGMA). Other primer criteria including polymorphism, primer efficiency and discriminatory values were calculated for each primer using different equations (Hunter and Gaston, 1988; Graham and McNicol, 1995).

$$GS = 2Nij\ (Ni+Nj)$$

Whereas,
GS = Genetic Similarity Index
Nij = The number of RAPD bands in common between genotypes i and j.
Ni and Nj = The total number of RAPD bands observed for genotypes i and j.
Genetic Distance (GD) = 1 - GS

SSR analysis

Photographs resulted from agarose gel electrophoresis were analyzed using Power Marker V.3 software (http://www.powermarker.net) for calculation of number of alleles, polymorphic information content (PIC) and heterozygosity. The distance method with program Power Marker V.3 was used for phylogenetic tree construction (Nei, 1972). Neighbore-joining method was used to obtain the tree.

RESULTS AND DISCUSSION

For current study, all the wheat genotypes were selected based on several features including protein content, good baking industry, lodging and disease resistance, resistance of breaking and shattering of spikes and slight variation in plant height. Thus genetic variation among these wheat genotypes proved using both RAPD and SSR markers, which is an important tool used by breeders in management of breeding programs to select the appropriate parental genotype for hybridization to secure the desirable heterosis in wheat.

RAPD markers analysis

The genomic DNA extracted from the fresh leaves of wheat genotypes and was examined through agarose gel electrophoresis. The DNA bands appeared with high intensity which illustrated their high molecular size (Figure 1). Both concentration and purity were 79.91 and 1.9 µg/ml, respectively.

Results RAPD markers analysis further revealed that molecular size of amplified fragments ranged from 110 (primer OPC-01) to 1648 bp (primer OPA-15) (Table 2). The highest value for main bands (12 bands) was produced by primer OPB-07. Higher numbers of monomorphic, polymorphic and unique bands were ten, three and two as shown by primers OPA-02, OPC-09, and OPB-07, respectively. Highest values for polymorphism, efficiency and discriminatory were 37.5, 0.065 and 42.8, respectively manifested by primer OPC-09. Primer OPC-09 was found as the most efficient one among the used primers and it produced highest number of polymorphic bands. Higher value for amplified bands (70) was produced by primer OPA-02. Polymorphic bands affect the marker polymorphism, efficiency and discriminatory values. However, the primers OPA-02, OPA-10, OPA-14, OPA-15, and OPF-05 were less efficient among the studied RAPD primers and failed to show the unique bands and fingerprinting for all the studied wheat genotypes (Figures 2, 3 and 4).

Results further illustrated that all the wheat genotypes were grouped in one large cluster except the genotype Dujlah, and the large cluster was divided between two sub-clusters, the first large one includes the wheat genotypes i.e., Tamuz-2, AL-Noor, Baghdad and Abu-Ghraib, while the other small cluster involves the genotypes Sham-6 and Tamuz-3 (Figure 5). The said arrangement of the wheat genotypes was not concerned with their collection site, which might be related to the genotypes shared same pedigree. New hybridization program should be accomplished to broaden the genetic base of Iraqi wheat genotypes. The higher genetic distance (0.3356) was recorded between the wheat genotypes i.e., Tamuz-2 and Dujlah, while the lowest genetic distance of 0.077 was observed between genotypes AL-Noor and Baghdad (Table 3).

Amplification profile using RAPDs showed high similarity index (86%) which was related to high monomorphic bands as compared to low polymorphic bands, which illustrated that the studied wheat genotypes belongs to same species represented through sharing some genome sequences. These identical sequences were constant in the rice genome and commonly referred as conserved sequences (Al-Judy, 2004). Al-Ghufaili and Al-Tamimi (2018) reported the RAPDs markers ability in revealing genetic variation in wheat germplasm might result from diverse germplasm and different primers (Qadir *et al*., 2015). Variation in molecular size of amplified products may be concerned with mutation (insertions and deletions) which may change in annealing sites of primers and result in change in size of amplified fragments and products. Changes in the distance between annealing sites of primers on template DNA, increase and decrease in number of main and amplified bands is directly related to the variation in number of

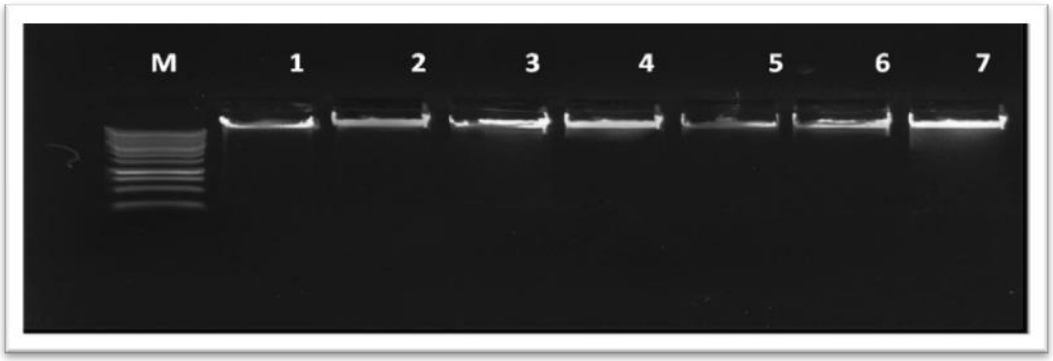

Figure 1. Agarose gel electrophoresis of genomic DNA: M: 1Kb DNA ladder and wheat genotypes, 1-Sham-6, 2-AL-Noor, 3-Tamuz-2, 4-Tamuz-3, 5-Abu-Ghraib, 6-Baghdad, and 7-Dujlah, using 1% agarose at 90 V for an hour.

Table 2. Summarized results of RAPDs amplification analysis.

Primers	Fragment size (pb)	Main bands	Amplified bands	Monomorphic bands	Polymorphic bands	Unique bands	Polymorphism	Efficiency	Discriminatory value
OPA-02	214-1356	10	70	10	0	0	0	0	0
OPA-10	311-1057	7	49	7	0	0	0	0	0
OPA-14	213-1476	7	49	7	0	0	0	0	0
OPA-15	252-1648	6	42	6	0	0	0	0	0
OPB-07	198-1068	12	66	8	2	2	16.6	0.03	28.5
OPC-01	110-1212	10	67	9	1	0	10	0.01	14.2
OPC-08	291-1008	7	38	5	1	1	14.2	0.026	14.2
OPC-09	337-1452	8	46	5	3	0	37.5	0.065	42.8
OPF-05	381-1159	5	35	5	0	0	0	0	0

Table 3. Genetic distance among seven wheat genotypes.

Genotypes	Sham-6	Al-Noor	Tamuz-2	Tamuz-3	Abu-Ghraib	Baghdad	Dujlah
Sham-6	0						
Al-Noor	0.2037	0					
Tamuz-2	0.1088	0.2037	0				
Tamuz-3	0.154	0.13337	0.10889	0			
Abu-Ghraib	0.2037	0.154	0.13337	0.13337	0		
Baghdad	0.1886	0.077	0.10889	0.1886	0.17218	0	
Dujlah	0.2982	0.154	0.2553	0.3356	0.308	0.1721	0

annealing sites recognized by primers which dependent mainly on target DNA sequence in wheat (Powell *et al*., 1996; Fadoul *et al*., 2013). Target DNA sequence mostly changed due to mutation that alters the primer annealing site and affects the number of both main and amplified bands (Williams *et al*., 1990; Tahir, 2014).

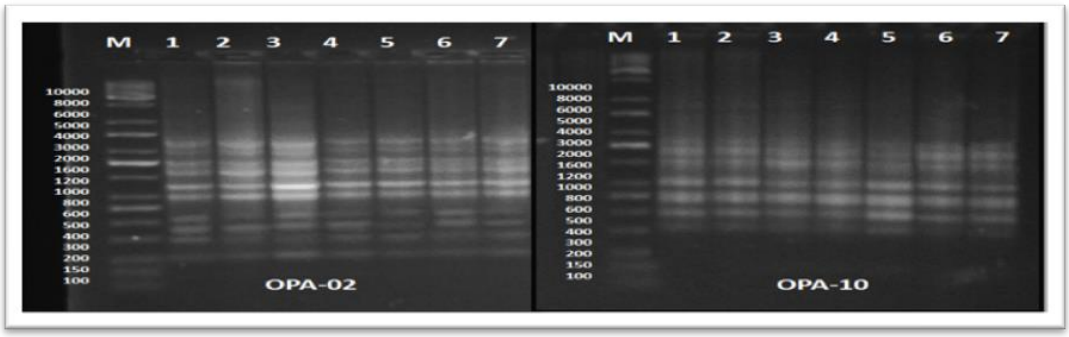

Figure 2. Gel electrophoresis for RAPD markers, OPA-02, and OPA-10 among the wheat genotypes, 1-Sham-6, 2-AL-Noor, 3-Tamuz-2, 4-Tamuz-3, 5-Abu-Ghraib, 6-Baghdad, and 7-Dujlah.

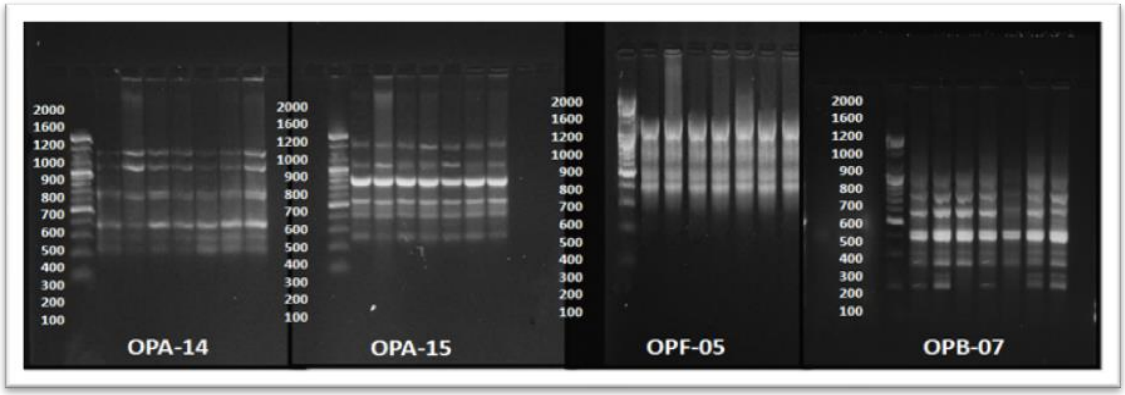

Figure 3. Gel electrophoresis for RAPD markers, OPA-14, OPA-15, OPF-05, and OPB-07 among the wheat genotypes, 1-Sham-6, 2-AL-Noor, 3-Tamuz-2, 4-Tamuz-3, 5-Abu-Ghraib, 6-Baghdad, and 7-Dujlah.

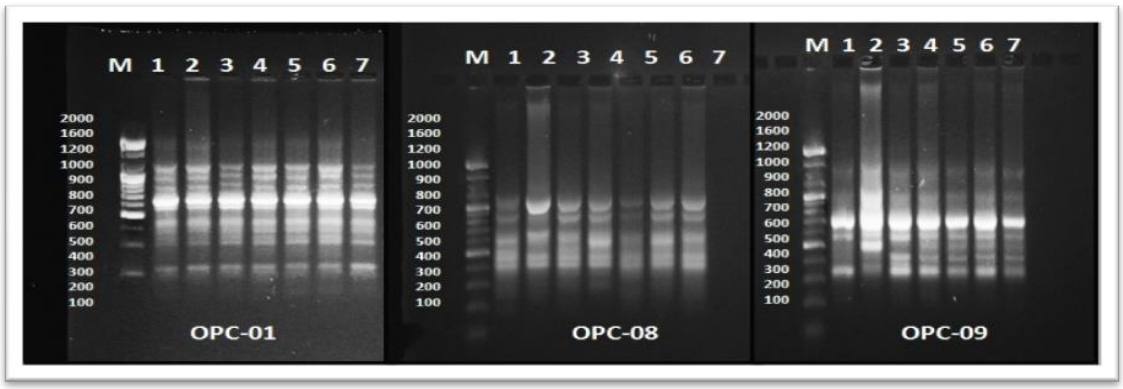

Figure 4. Gel electrophoresis for RAPD markers, OPC-01, OPC-08, and OPC-09 among the wheat genotypes, 1-Sham-6, 2-AL-Noor, 3-Tamuz-2, 4-Tamuz-3, 5-Abu-Ghraib, 6-Baghdad, and 7-Dujlah.

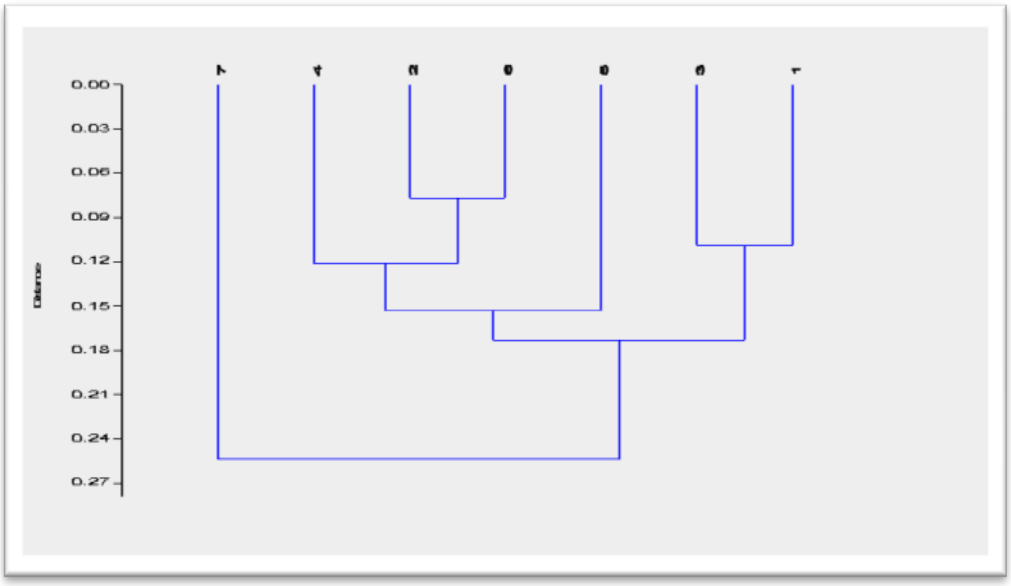

Figure 5. Phyllogenetic relationship among the wheat genotypes, 1-Sham-6, 2-AL-Noor, 3-Tamuz-2, 4-Tamuz-3, 5-Abu-Ghraib, 6-Baghdad, and 7-Dujlah using RAPDs.

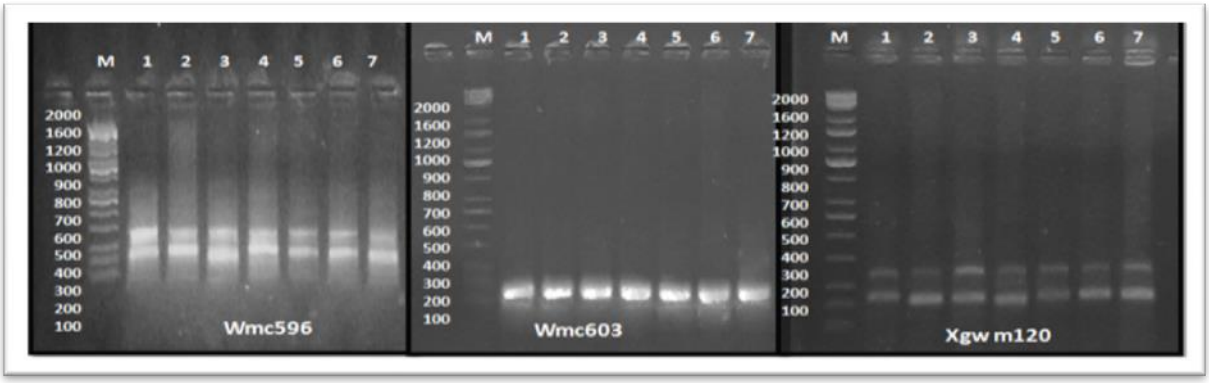

Figure 6. Gel electrophoresis for SSR markers, Wmc596, Wmc603 and Xgwm120 in wheat genotypes, 1-Sham-6, 2-AL-Noor, 3-Tamuz-2, 4-Tamuz-3, 5-Abu-Ghraib, 6-Baghdad, and 7-Dujlah.

The presence of unique bands in two RAPD primers OPB-07 and OPC-08 reflects their ability to recognize a unique annealing site on template. This affects primers ability to produce a unique DNA fingerprint for a particular wheat genotype (Fadoul *et al*., 2013; AL-Tamimi, 2014; Al-Ghufaili, 2017). Genetic distance and arrangement among maize genotypes in phylogenetic tree were not concerned with genotypes pedigree and collection sites (AL-Saadi, 2018).

Efficiency of primer OPC-09 disagree with findings of Al-Ghufaili and Al-Tamimi (2018) in wheat, however, agree with Thamir *et al*. (2014) on tomato which might be due

to use of diverse germplasm. High efficiency of primer OPC-09 was related to its ability to produce high polymorphic bands (Hunter and Gaston, 1988; Graham and McNicol, 1995). However, the highest genetic distance refers to low similarity between two wheat genotypes and its preferable character to get heterosis in wheat.

SSR markers analysis

The SSR markers produced fragments ranging between 123 to 361 bp. The number of alleles obtained per locus by different primers were varied i.e., one each allele in three SSR primers (Wmc603, Xgwm608, and Xgwm639), two each alleles in three SSR primers (Wmc596, Xgwm120, and Xgwm264), and three alleles in primer Xgwm1623A (Figure 6). In SSR primers, the heterozygosity ranged from 0.00 (Wmc6037A, Xgwm608 and Xgwm6395B) to 1.00 (Wmc596, Xgwm1202B and Xgwm2641B). The highest genetic diversity (0.5850) and polymorphism information content (0.4949) were produced by SSR primer Xgwm1623A. Major alleles frequency ranged between 0.45 (Xgwm1623A) to 1.00 (Wmc6037A, Xgwm608, and Xgwm6395B). The most efficient SSR primer was Xgwm1623A while the least efficient SSR primers were Wmc6037A, Xgwm608, and Xgwm6395B (Table 4, Figure 7). Among all used SSR markers, the primer Xgwm1623A produced the highest values for allele number (3), gene diversity (0.5850) and PIC (0.4949). Among seven wheat genotypes, the six genotypes (Sham-6, AL-Noor, Tamuz-2, Abu-Ghraib, Baghdad and Dujlah) were grouped in one large cluster except Tamuz-3 (Figure 8).

Amplification profile using SSRs resulted in PIC value (0.4945) by primer Xgwm1623A which recognized it as highly informative primer in studying genetic diversity among wheat genotypes (Botstein *et al*., 1980). The PIC value determines high informative SSRs loci and its ability in detection of variation among the maize genotypes based on their genetic relationship (Legesse *et al*., 2007; Rajendran *et al*., 2014).

Heterozygosity can be defined as the proportion of heterozygous individuals in a population at a single locus (Botstein *et al*., 1980). It could provide an idea of the information available from the SSRs loci for detectable variation among the wheat lines based on their genetic relationship which mainly concerned mainly to the variation in their genetic base (Eyherabide *et al*., 2006; Morales *et al*., 2010). Gene diversity which referred to the expected heterozygosity, is defined as the probability of two randomly chosen alleles from a population to be different (Liu and Muse, 2005), and its value increases as polymorphism increases. Allele number varies according to number of maize genotypes, and their origin used in the study (Kamalesh *et al*., 2009; Gurung *et al*., 2010; Babu *et al*., 2012).

Highest values for major allele frequency were produced by primers Wmc6037A, Xgwm608 and Xgwm6395B. Generally, the values of major allele frequency enhanced as the homozygosity increased and the same also reported in these primers. Genotypes arrangement in phylogenetic tree was not concerned with genotypes pedigree and collection origins (Al-Saadi, 2018). Construction of SSR markers phylogenetic tree illustrated the high similarity as

Table 4. Summarized results of seven SSR markers analysis.

Markers	Molecular size range (bp)	Allele number	Major allele frequency	Gene Diversity	Heterozygosity	PIC
Wmc596	186-277	2.0000	0.5000	0.5000	1.0000	0.3750
Wmc603-7A	123	1.0000	1.0000	0.0000	0.0000	0.0000
Xgwm120-2B	168-244	2.0000	0.5000	0.5000	1.0000	0.3750
Xgwm162-3A	239-361	3.0000	0.4500	0.5850	0.9000	0.4949
Xgwm264-1B	188-259	2.0000	0.5000	0.5000	1.0000	0.3750
Xgwm608	174	1.0000	1.0000	0.0000	0.0000	0.0000
Xgwm639-5B	146	1.0000	1.0000	0.0000	0.0000	0.0000

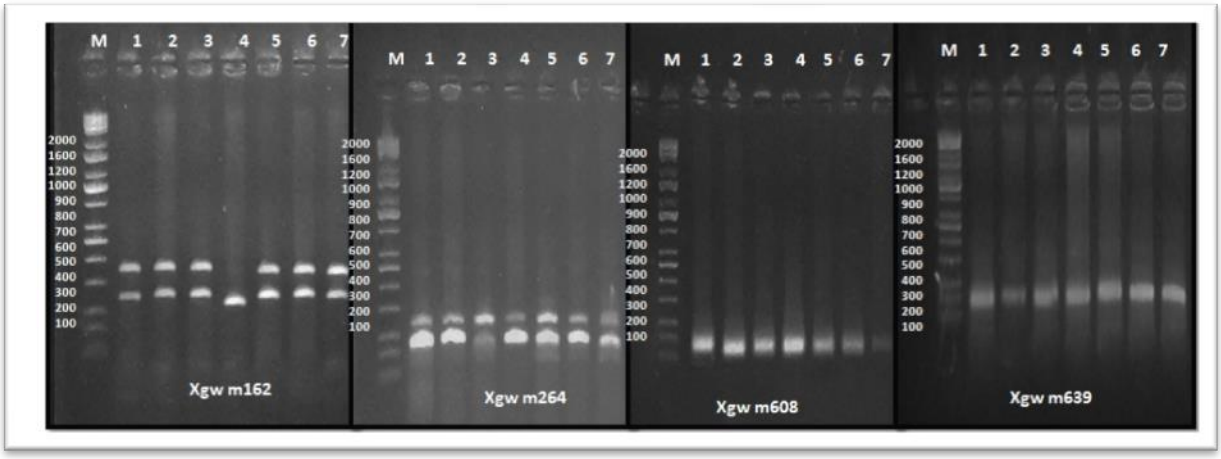

Figure 7. Gel electrophoresis for SSR markers, Xgwm1623A, Xgwm264, Xgwm608 and Xgwm639 in wheat genotypes, 1-Sham-6, 2-AL-Noor, 3-Tamuz-2, 4-Tamuz-3, 5-Abu-Ghraib, 6-Baghdad, and 7-Dujlah.

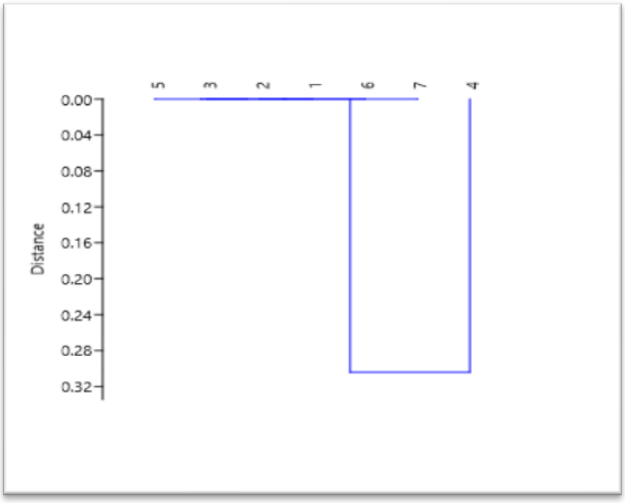

Figure 8. Phyllogenetic relationship among the wheat genotypes, 1-Sham-6, 2-AL-Noor, 3-Tamuz-2, 4-Tamuz-3, 5-Abu-Ghraib, 6-Baghdad, and 7-Dujlah.

revealed by these markers which enunciated that these wheat genotypes share similar pedigree, and in other words, it may referred to narrow genetic base of the Iraqi wheat germplasm. Therefore, there is an intensive need to broaden the genetic base of wheat, as increased genetic variation is important for successful hybridization in breeding to get desirable heterosis. Rauf *et al*. (2010) reported that reduction in genetic diversity always resulted from the fact that plant populations are subjected to both domestication and pedigree selection during pre-systematic plant breeding.

Positive correlation was observed between gene diversity and allele number as appeared by using the primer 1623A, and these results got support from the past findings as reported by Islam *et al*. (2012). The SSR markers proved their ability to distinguish among the wild species of wheat possess genomes A, D and C (Salehi *et al*., 2018) and wheat cultivars in same species (Yadav *et al*., 2019). Such type of marker assisted selection studies is efficient to replace conventional breeding approaches with molecular breeding.

CONCLUSION

Both RAPD and SSR markers effectively revealed genetic diversity among the studied wheat genotypes. However, there is an intensive need to increase the genetic variation and broaden the genetic base of Iraqi bread wheat germplasm for more successful breeding to get desirable heterosis in wheat.

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