Iraqi Journal of Biotechnology, April 2020, Vol. 19, No. 1, 1-7

Estimation of Genetic Variation and Distance Between Selected Wheat Genotypes and local Cultivars

Eman N. Ismail[1] , Ali A. Al-Salihy[2] , Ibrahim I. H. Al-Masshadani[1]

[1]Biotechnology Research Center/ Al-Nahrain University
[2]Institute of Genetic Engineering and Biotechnology/ University of Baghdad

Received: December 1, 2019 / **Accepted:** March 16, 2020 / **Published:** April 28, 2020

Abstract: Molecular studies were carried out to estimate genetic variation and genetic distance in selected genotypes (Furat**,** 4H, 7H) and two local cultivars (Iraq, Orok) wheat. DNA was extracted from plant leaves of these genotypes and cultivars at potting stage. The PCR reaction method was used to estimate the genetic variation. Six primers were used in this study. Data of polymorphic fragment were analyzed to determine the genetic relationship between the genotypes and cultivars, and also genetic distance was estimated. Bands differed between cultivars in number and molecular weight which appeared in these genotypes and cultivars using six primers exhibition genetic variation. Results of the dendrogram showed that the selected genotypes and cultivars are divided into three groups according to the genetic variation between them. Results also indicated that the local cultivars and selected genotypes differed in their genetic distance.

Keywords: wheat, PCR, RAPD, genetic variation.

Corresponding author: (Email: emandi84@yahoo.co.uk).

Introduction

Wheat genotypes and cultivars were deferred in their growth and productivity according to the genetic variations between them. Soil physical and chemical compounds are affect the growth and yield production of whet plants (1). The degree of these effects depends on kind of genotypes and cultivates. Plant breeding and improvement programs are important to induce new genotypes with high genetic variation in their growth and production (2, 3). Increasing plant production through plant breeding depends on genetic variation in the germplasm for growth and productivity (4). Improvement cultivars or genotypes with high production and quality is an attractive for increasing the yield production under field condition (2,4,5). Genetic improvement in any characters was more correlated with the genetic variation in genetic material which will be used in plant breeding and improvement programs. This genetic variation depends on the genetic distance between the parents that used in hybridization program. Previously, the genetic distance between the selected genotypes will be determined by PCR-RAPD. They found high genetic distance between the selected genotypes and local cultivars, but less geneic distance was found between the selected genotypes (2, 3, 6)

The aim of this study is to estimate of genetic variation and distance between some wheat genotypes which selected through plant breeding program by using PCR-RAPD technique.

Materials and methods

Selected wheat genotypes

Selected wheat genotypes (Furat, 4H, and 7H) were selected through plant breeding and improvement programs (4,7) and two local cultivars were used in this study.

Field experiment

Seeds of selected wheat genotypes (Furat, 4H, 7H) and two sensitive cultivars (Iraq and Orok) were sown in soil at the rate of 10 seeds for each genotype and for each pot and placed under plastic house then irrigated with 500 ml of tap water. Three Leaf samples were cut at potting stage for DNA extraction.

DNA Extraction

DNA was extracted using kit from (Hamorabi biotechnology production laboratory) / Institute of genetic Engineering & Biotechnology for post graduate studies / University of Baghdad. The DNA was verified after separation by electrophoresis on a 1.5% agarose gel containing 2µl red safe stain and visualized under UV light. DNA Purity was measured by using nanodrop.

RAPD assay

The primer were supplied by Bioneer-Korea and used in RAPD (Table 1). The PCR reaction mixture consisted of PCR pre mix 1x (Bioneer-Korea) 5 µl, Deionised D.W 11 µl, primer (10pmol /µl) 2 µl and DNA (100 µl) 2 µl. The thermal cycling profile consisted of initial denaturation at 95^{o}c for 3 min and 40 cycles at 95^{o}c for 1 min, 35^{o}c for 1 min and 72^{o}c for 1 min with a final extension at 72^{o}c for 10 min. Amplification products were analyzed by 1.5% agarose gel electrophoresis containing red safe stain and visualized under UV light. The ladder was 100bp (Intron/Korea).

Table (1): RAPD primers used in PCR reaction

Primer	Sequence
OP-M14	AGGGTCGTTC
OP-R06	GTCTACGGCA
OP-K01	CATTCGAGCC
OP-V19	GGGTGTGCAG
OP-M20	AGGTCTTGGG
OP-V14	AGATCCCGCC

Estimation of genetic distance

Data of PCR reaction were analyzed to estimate the genetics distance. The amplification profiles of all isolates were compared, the presence was scored as (1) and the absence of the same band of the same size in other generation scored as (0). Amplified fragments were analyzed for genetic relationship analysis. Estimates of genetic distance between the selected genotype and local cultivar of wheat were calculated based on following formula: G.D =1- {2Nab/ (Na + Nb)} (8), where Na= the total number of

fragments detected in individual (a); Nb= the total number of fragments shown by individual (b) and Nab= the number of fragments shared by individuals (a) and (b). Cluster analysis was done to determine genetic relationship tree diagrams among genotypes and cultivars. All computations were carried out using the Numerical Taxonomy and Multivariate Analysis System (NTSYSpc), Version 1.7 package (9).

Results and discussion

Genetic variations

A- Primers OP-M14 and OP-R06

The results in (Figure 1, Table 2) showed the bands that appeared by OP-M14 and OP-R06 primer. The OP-M14 primer gave six (6) bands at different molecular weight (3500-550bp). This primer exhibited 5 band in Furat cultivar, 4H and Iraq cultivar were similarly in the same molecular weights whilst, appeared six (6) band, in the 7H genotype and Orok cultivar with the same molecular weight, but the Furat cultivar, 4H and cultivar Iraq were differed with the 7H genotype and cultivar Orok in one band that molecular weight (775 bp). Also the results in (Figure 1, Table 2) revealed that OP-R06 primer gave 5 bands at different molecular weights (900-375bp). The Furat cultivar was similar with the 4H and 7H genotypes at three bands at the same molecular weight, while them differed with Iraq cultivars at one band (550bp), and also differed with Orok cultivar at two bands (900,550bp).

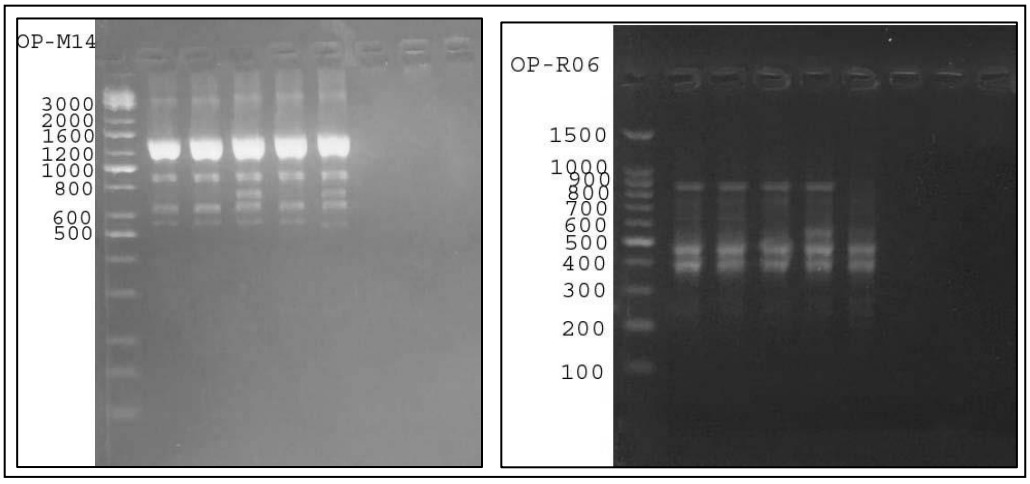

Figure (1): PCR product of OP-M14 and OP-R06 primer the product was electrophoresis on 1.5% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. DNA ladder (100).

B- Primers OP-K01 and OP-V19

The result of OP-K01 primer showed that this primer gave four (4) bands with different molecular weights, these were similar in all studied genotypes (Furat, 4H, 7H, and Iraq) except the Orok cultivar which differed from them in all appeared bands (Figure 2, Table 2). One band with molecular weight (500bp) did not appeared in Furat, 4H, 7H genotypes and Iraq cultivar, while appeared only in Orok cultivar. Also, the results indicated that

the OP-V19 primer gave five bands with molecular weights (800, 575, 450, 400, 350) bp. Furat and Orok cultivars were similar in four that appeared by OP-V19 primer, while 4H genotype was similar with them, but differed with them cultivars in one band with molecular weight (800bp) (Figure 2, Table 2). The Iraq cultivar and 7H genotype was differed on one band (400bp) with the others genotypes.

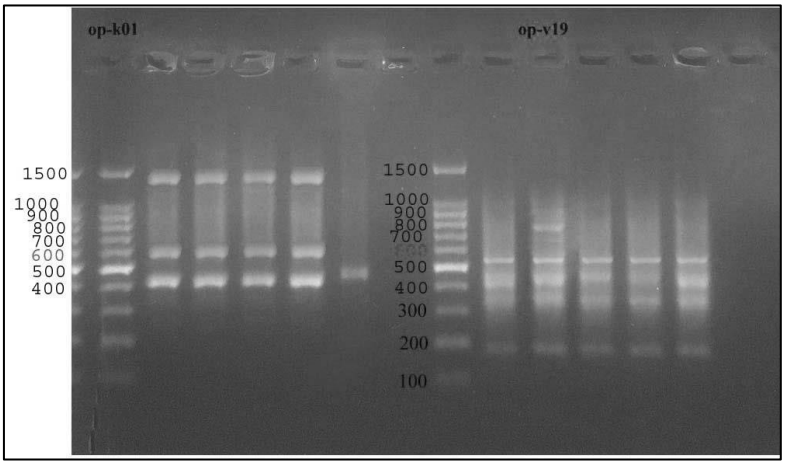

Figure (2): PCR product of OP-K01 and OP-V19 primer the product was electrophoresis on 1.5% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. DNA ladder (100).

C- Primers OP-M20 and OP-V14

The results in (Figure 3, Table 2) indicated that primer OP-M20 gave seven (7) bands at different molecular weight ranged from 1000 bp to 275bp. There are differences between the genotypes and cultivars in number of appearance bands, one band (1000bp) in Furat cultivar, four bands in 4H genotype, three (3) bands in 7H genotype and Iraq cultivar, and seven bands in Orok cultivar (Figure 3, Table 2) The Furat cultivar differed only with 4H genotype in one band (650bp) and Orok cultivar in 4 bands (650,400,325 and 275 bp).also the number of bands that gave by OP-V14 primer were seven bands all of them appeared in Furat cultivar, six bands were appeared in 4H and 7H genotypes, four bands appeared in Iraq cultivar, and three bands appeared in Orok cultivar (Figure 3, Table 2). The results also showed that Furat cultivar was differed with 4H and 7H genotypes only in one band with molecular weight 300bp, while its differed with Iraq cultivar in three bands and with Orok cultivar in four bands. The lower molecular weight bands (250,200) bp were appeared in selected genotypes, while they absent in local wheat cultivars (Iraq, Orok) (Figure 3, Table 2). The Furat cultivar and 4H, 7H genotypes were selected through exposure of selection program. In the previous studies indicated that these genotypes and other selected genotypes by this program differed in their growth behavior and morphological as compared with the local cultivars which plant in Iraq in normal soils (2, 4, 7, 10). Also the results which reported by (11), showed the growth and production of some selected genotypes were differed according to the soil type.

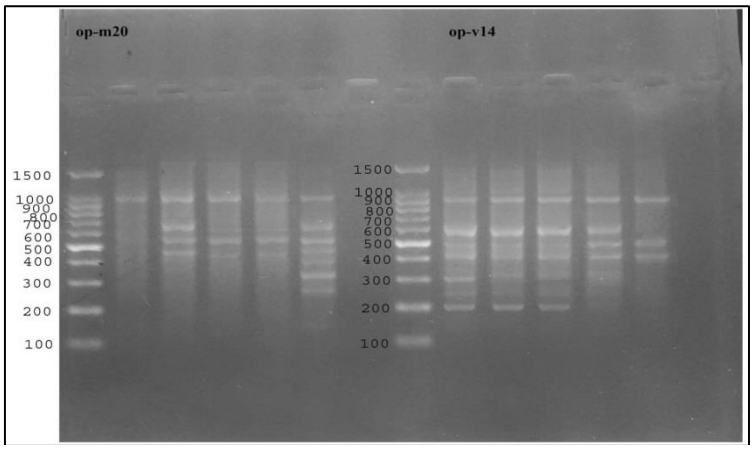

Figure (3): PCR product of OP-M20 and OP-V14primer the product was electrophoresis on 1.5% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. DNA ladder (100).

Table (2): RAPD-PCR product of six primers on 1.5% agarose gel- 100 bp DNA ladder.

PRIMER	M.W.	Furat	4H	7H	Iraq	Orok
OP-M14	3500	1	1	1	1	1
	1200	1	1	1	1	1
	900	1	1	1	1	1
	775	0	0	1	0	1
	650	1	1	1	1	1
	550	1	1	1	1	1
OP-R06	900	1	1	1	1	0
	550	0	0	0	1	0
	475	1	1	1	1	1
	375	1	1	1	1	1
OP-K01	1400	1	1	1	1	0
	600	1	1	1	1	0
	500	0	0	0	0	1
	425	1	1	1	1	0
OP-V19	800	0	1	0	0	0
	575	1	1	1	1	1
	450	1	1	1	1	1
	400	1	1	0	0	1
	350	1	1	1	1	1
OP-M20	1000	1	1	1	1	1
	650	0	1	0	0	1
	550	0	1	1	1	1
	450	0	1	1	1	1
	400	0	0	0	0	1
	325	0	0	0	0	1
	275	0	0	0	0	1
OP-V14	900	1	1	1	1	1
	600	1	1	1	1	0
	500	1	1	1	1	1
	400	1	1	1	1	1
	300	1	0	0	0	0
	250	1	1	1	0	0
	200	1	1	1	0	0

The aim of this study is estimation of genetic distance between new induced genotypes and local wheat cultivars by using RAPD-PCR. Six primers (OP-M14, OP-R06, OP-K01, OP-V19, OP-M20 and OP-V14) were

used to finding bands to determent the variation between the cultivars and genotypes to detect if genetic variations exist between them. The results of banding patterns indicated that the primers were differed between them in number of bands and their molecular weights. All the primers appeared in different cultivars and genotypes this indicate the annealing of primers to similar DNA regions in these cultivar and genotypes studied that might be conserved regions in wheat DNA (12), and also found the same results which reported in this study. Differences between the selected genotype and local cultivars due to the variation between them in morphological characters, so these genotypes were selected by plant breeding and improvement program (4, 7, 10). Bands which appeared common in some selected genotypes and cultivars of wheat may also prove important as they can be used by linking it to traits that are shared is these genotypes and cultivars such as tolerance pathogens, phenotype characters or environmental stress (13). The differences between the selected genotypes in some bands may be referring to genetic variation between them in growth characters which determined in the previous studies (2, 3, 4, 7).

Genetic distances

The results which reported in Figure (4) showed that the selected genotypes and cultivars of wheat divided to the three groups, first group included Furat cultivar, 4H and 7H genotypes, second group include only Orok cultivar, and third group included also only Iraq cultivars. The value of genetic distance revealed that there is distance between induced genotypes and local cultivars (Table 3). Genetically, there are close similarly between the selected genotypes (Furat, 4H and 7H) as compared with the local cultivars. Also, the local cultivars also were closing similar in their genetics distance. The large genetic distance between the selected genotypes and local cultivars may be due to differences between them morphological characters. The selected genotypes were similar in most bands which appeared by the six primer, while they differed in most bands with the local cultivars, especially with the Iraq cultivars, because they are differed in their production and morphological characters. Previously, studies of genotypes providing useful information as these genotypes differed in their growth behavior to produced fingerprints (12).

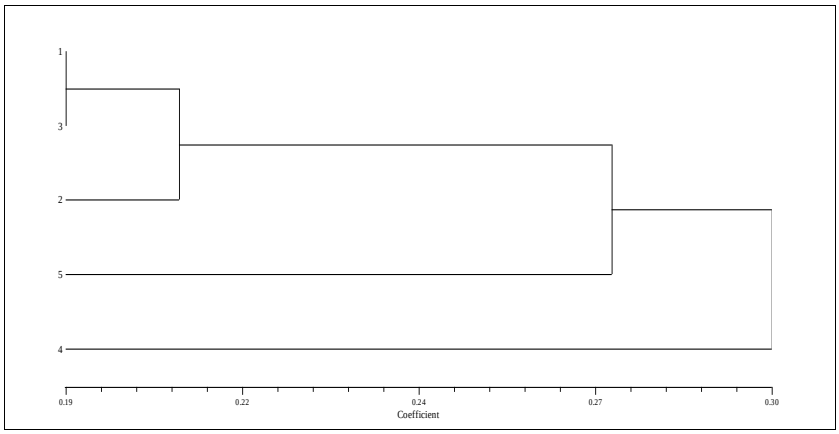

Figure (4): Dendrogram illustrated genetic fingerprint and relationships between wheat cultivar developed from RAPD data. Furat (1) ,4H(2), 7H (3), Iraq(4), Orok (5).

Table (3): Values of genetic distance between wheat cultivar calculated according to Nei & Li's, (1979).

orok	Iraq	7H	4H	Furat	Genotypes
				0	Furat
			0	0.19626	4H
		0	0.2	0.18095	7H
	0	0.24211	0.27835	0.30435	Iraq
0	0.36538	0.21368	0.29412	0.31579	Orok

Generally, there are genetic variation within genotypes (Furate,4H and 7H) and local cultivars and more genetic distance between them may be refer to the differences between them in morphological characters, and in yield production.

References

1. Maas, E.V. and Hoffman, G.J. (1977). Crop Salt Tolerance-Current Assessment. *Journal of the Irrigation and Drainage Division*, 103, 115-134.
2. Al-Mishhadani, I. (2015). Estimation of salt tolerance degree in some selected wheat genotypes by using detection of salt tolerant gene (*TaSTK*) and its expression under salinity condition. *International Journal of Applied Agricultural Sciences*, 1(2): 31-35.
3. Al-Mishhadani, I. H. I.; Majeed, D. M.; Ismail, E. N. and Kadhim, M. K. (2016). Detection of Salt Tolerant Gene (*TaNIP*) and Its Expression in Three Selected Wheat Genotypes Through Plant Breeding Programs Under Salinity Conditions. I*nternational Journal of Applied Agricultural Sciences*, 2(1): 12-16
4. Al-Mishhadani, I. H. I. (2012). Breeding and Selection of Some Lines of Bread Wheat for Salt Tolerance. *Journal of Agriculture Science and Technology B*., 12(8B):934.
5. Epstein, E.; Norlyn, J.D.; Rush, D.W.; Kingsbury, R.W.; Kelley, D.B.; Cunningham ,G.A., *et al*. (1980). Saline culture of crop: A genetic approach. *Science*, 210: 399-404.
6. AL-Salihy, A.A. and Jabbar , M. Kh. (2017). " In Vitro: Detection TaSOS1 gene in four Iraq genotypes of Bread Wheat under different salt stress levels. *Iraqi Journal of Biotechnology*, 16(4): 69-78.
7. Al-Mishhadani, I. H. I.; Ismail, E. N.; Jaddoa, K. A.; Majeed, D. M. and Mohammed, O. A. (2015). Estimation of the Interaction Effect Between Salinity and Growth Regulators on Salt Tolerance of Two Bread Wheat Cultivars. *International Journal of Applied Agricultural Sciences*, 1(4): 95-101.
8. Nei, M. and W. H.Li. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences*, 76(10): 5269-5273.
9. Rohlf, F. J. (1993). NTSYS-PC: Numerical Taxonomy and Multivariate Analysis System. Version 1.7. Applied Biostatistics Inc., Steauket, New York. USA.
10. Al-Mishhadani, I. H. I. (2010). Response of some newly developed salt- tolerant genotypes of wheat to naturally salinized soil. The 5th Int. Conf. for Develop. And the Env. In the Arab world. 21-23.
11. Al-Mishhdany, I. I. H. and Mohamed, L. Sh. (1999). Yield components comparison and correlation in nine genotypes of wheat under saline conditions. *Ibn Al-Haitham J. for pure and App. Sci*., 10(2): 10-20.
12. Zahid, R. A.; Al-Mishhadani, I. H. I.; Hammed, S. N.; Majeed, D. M.; Aarf, G. L. and Majeed, S. M. (2011). Evaluation of Random Amplified Polymorphic DNA as a Genetic Indicator of Salt Tolerance in Iraqi Wheat. *Tikrit Journal of Pure Science*, 16 (4).
13. Rus-Kortekaas, W.; Smulders, M.J.M.; Arens, P. and Vosman, B. (1994). Direct comparison of levels of genetic variation in tomato detected by a GACA-containing microsatellite probe and by random amplified polymorphic DNA. *Genome*, 37(3): 375-381.