*Plant Archives* Vol. 19 No. 2, 2019 pp. 3607-3612 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

# CREATION OF MOLECULAR DATABASES TO IRAQI BREAD WHEAT CULTIVARS

**Ali S. Mueen and Mundher Kh. Jabbar***

Plant Genetics and breeding Lab., University of Al-qasim Green, Babil, Iraq.

## Abstract

Seventeen Iraqi bread wheat cultivars developed through plant breeding programs were screened for getting molecular databases through RAPD marker. A total of 799 loci were amplified with 10 primers out . Fragments size ranged from 200bp-1.8kb and fragments produced by various primers ranged from 1-10 with an average of 5.3 fragments per primer. The highest number of bands (111) was amplified with primer OP-M06 while the lowest number (39) with primer OP-K01. Results were revealed that variety Baghdad1 gave low number of band values in OP-M06, Muhood was gave low band values in OP-M14 primer, in OP-M20 primer, the similarity was found among Baghdad 3, Alez and Iraq , another similarity was found among Babil 113, furat and Buhooth 158, the OP-I02 primer was given higher number of bands in aboghreeb cultivar while low number of bands in Latifia, the similarity was between Babil113 and Sham 6, Baraka and Buhooth 10 were similarity too, OP-V02 primer did not give clear variations but Buhooth 22 characterized by giving low bands, OP-V19 was not given variations and characterized, OP-K 01 primer was given low band number in Latifia, OP-M 14 primer was gaven similarity among Buhooth 22, Latifia and Buhooth 158, another similarity was among Furat, Baghdad1 Alez, Babil 113 cultivar was similar Buhooth 22 in Op-P 04 primer, there were similarity and differences between two groups of cultivars in OP-H 01primer.

***Key words:*** Bread wheat, genetic database, Molecular, RAPD markers.

## Introduction

Every year appear new cultivars and breeds to different plant species for improving of the production and tolerance of stresses (Jabbar, 2019 and Guzman *et al.,* 2018), these new cultivars come from the interring, hybridization, gene cloning and ... *etc*., to discrimination among new genotypes and registered cultivars, it need databases include either plant or genetic traits (AL-Salihy *et al.,* 2018 and Balkan, 2018), to get plant traits, it need sow seeds and study of plant traits in throughout the season, so this method need long time, study of genetic traits has been a quick and accurate results (AL-Salihy and Jabbar, 2017 and Khan *et al.,* 2017), to compare new genotype with registered cultivars, it need genetic or molecular databases.

Recently, molecular parameters have used to compare among the genotypes from use group of primes which certify from the research foundations after its study and certify, the primers have used for comparing with new genotypes (Noah, 2018).

***Author for correspondence :** E-mail : mukhjabbar@gmail.com

Molecular biology techniques lead to the development of DNA markers that have been effectively used to identify a number of features in various cultivars (Barakat *et al.,* 2010). In this target, the RAPD-PCR (Random Amplified Polymorphic DNA) assay is one of the most widely usedmethod and an easy PCR-based technique for producing molecular markers (Bhutta *et al.,* 2006). RAPD technology is an important tool for rapid identification of markers associated with the genetic variation among wheat genotypes (Ali *et al.,* 2013). RAPD analysis may be used as a method for detection of genetic polymorphism, and as a source for unique locus-specific markers.

So this study included use random amplified polymorphic DNA assay to create databases of Iraqi bread wheat cultivars and applied it in new genotype.

## Materials and Methods

The experiment was conducted in field of agriculture college and laboratories of biotechnology college– Al-qasim green university.

**Plant material**

17 Iraqi cultivars with 1 Iranian cultivar (for compare) of bread wheat were used in the present investigation to determine polymorphic molecular markers RAPD. Samples of 17 varieties were provided by different Institutes which were affiliated to ministry of higher education and ministry of agriculture compare cultivar was entered from Iran. the origin of cultivars were remembered in table 1. The green leaves were used, it was taken at booting stage.

**Oligonucleotide primers**

There were10-mer oligonucleotide primers were designed (Table 2) by primer design online program in center of biotechnology web , the primers were lyophilized, they were dissolved in the free dd $H_2O$ to give a final concentration of 100 pmol. $\mu l^{-1}$ as stock solution and keep a stock at -20 to prepare 10 pmol. $\mu l^{-1}$ concentration as work primer suspended, 10 µl of the stock solution in 90 µl of the free dd$H_2O$ water to reach a final volume 100 µl was investigated by IDT (Integrated DNA Technologies company, Canada).

**DNA extraction:** Total DNA was extracted from green leaves, Total genomic DNA was extracted according to the standard procedure of. (Spaniolas *et al.,* 2008) with some modifications byintron biotechnology/ Korea, type of PCR was thermocycler PCR, sample contents of thermocycler PCR for transcription in Table 2, the PCR program was remembered from (Vierling and Nguyen, 1992).

**PCR amplification and electrophoresis**

PCR amplification was performed in 1x PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 0.1% Triton) 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 25 pM of each primer, 10-50 ng of genomic DNA per 25, l of reaction volume and 2 units of Taq polymerase. The amplifications were carried out on a crocodile III (Appligene) programmed for one cycle of denaturizing at 94°C for 5 min. and 35 cycles of 30 s at 94°C, annealing of 1 min. at 60°C and extension of 1 min. at 72°C, a final extension of 7 min. at 72°C was done. PCR products were separated on 2% agarose gels, then stained with red safe Nucleic acid staining solution and scored for presence or absence of bands. Since RAPD markers are dominant, a locus was considered to be polymorph if the band was present in one pattern and not in the other (Noah, 2018).

**Calculate of molecular weight**

Photo cad program was used for calculating molecular weight from detection of the bands and compare with DNA ladder molecular weight (Cerasela *et al.,* 2011), RAPD results were analyzed by Convert metadata into numeric data (number 1 in band and 0 no band), the genetic distance coefficient was calculated from Nie´s equation (Nie and Lie, 1979):

$$\text{Genetic Distance} = 1 \quad \frac{2 \quad N_{xy}}{N_y \quad N_x}$$

$N_{XY}$ = number of collective bands between samples X and Y.

$N_X$ = number of total bands in X sample.

$N_Y$ = number of total bands in Y sample.

Linkage analysis was performed using the UPGMA program (Sneath and Sokal, 1973).

## Results

In Figs 1-10, the primers gave bands in all wheat cultivars , seven primers gave number of markers over 4. most primers gave wide range of markers from 200 bp to 1800 bp, the range arrived 1400bp (1800-400 = 1400) in Op-P04 primer compare to OP-V02 which gave low range 450bp, OP-M06 primer gave high number of markers, it gave high number of bands 111, six primers gave high number of bands over 80 (Table 3).

All cultivars gave bands in figs 1-10, Baghdad1 cultivar characterized in OP-M06 primer (P.1) by giving

**Table 1:** Origin's cultivars and genotypes analyzed.

| Origin's | Number of cultivars | Name of cultivars | Origin's | Number of cultivars | Name of cultivars |
|---|---|---|---|---|---|
| Iraqi cultivars | 1 | Babil 113 | Iraqi cultivars | Latifia | 9 |
| | 2 | Furat | | Baghdad1 | 10 |
| | 3 | Baghdad 3 | | Sham6 | 11 |
| | 4 | Buhooth 10 | | Ibaa 99 | 12 |
| | 5 | Tamuz 2 | | Alez | 13 |
| | 6 | Rashid | | Buhooth 158 | 15 |
| | 7 | Fares 1 | | Iraq | 16 |
| | 8 | Buhooth 22 | | Baraka | 17 |
| Iranian cultivar | 14 | Muhood | | Aboghreeb | 18 |

**Table 2:** the list of RAPD primer.

| No. | Primer | Sequence | No. | Primer | Sequence |
|---|---|---|---|---|---|
| 1. | OP-M06 | CTGGGCAACT | 6. | OP-V19 | GGGTGTGCAG |
| 2. | OP-R14 | CAGGATTCCC | 7. | OP-K01 | CATTCGAGCC |
| 3. | OP-M20 | AGGTCTTGGG | 8. | OP-M14 | AGGGTCGTTC |
| 4. | OP-I02 | GGAGGAGAGG | 9. | Op-P04 | GTGTCTCAGG |
| 5. | OP-V02 | AGTCACTCCC | 10. | OP-H01 | GGTCGGAGAA |

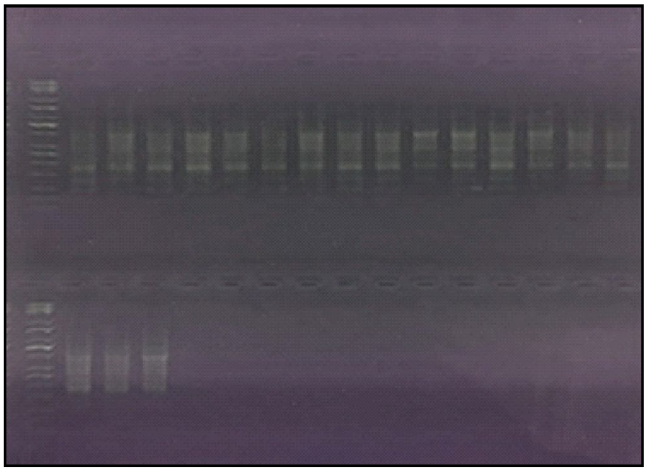

**Fig. 1:** PCR product of OP-M06primer The product was electrophoresis on 2% agarose at 5 volt/cm$^2$. 1x TBE buffer for 1:30 hours. N: DNA ladder (100).

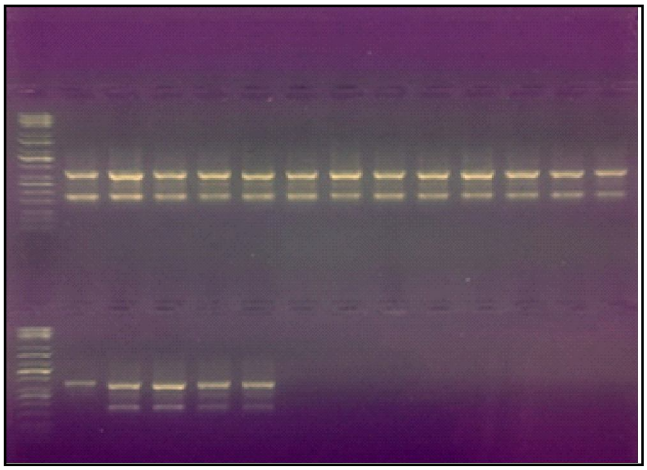

**Fig. 2:** PCR product of OP-R14primer, The product was electrophoresis on 2% agarose at 5 volt/cm$^2$. 1x TBE buffer for 1:30 hours. N: DNA ladder (100).

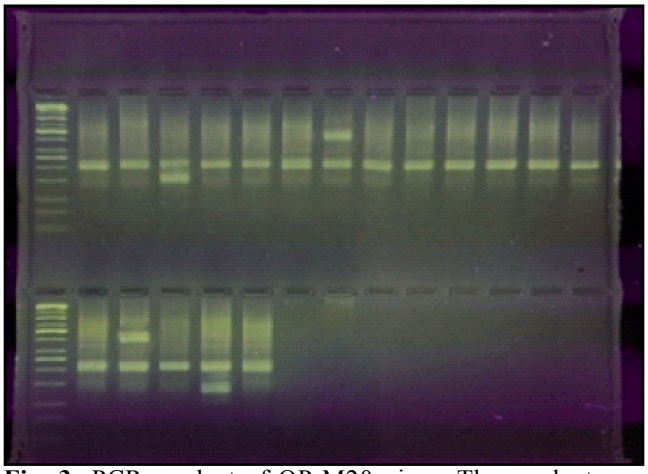

**Fig. 3:** PCR product of OP-M20primer The product was electrophoresis on 2% agarose at 5 volt/cm$^2$. 1x TBE buffer for 1:30 hours. N: DNA ladder (100).

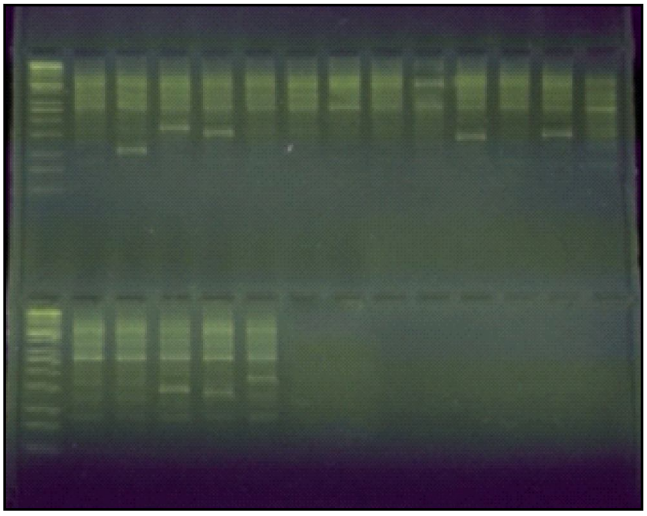

**Fig. 4:** PCR product of OP-I02 primer The product was electrophoresis on 2% agarose at 5 volt/cm$^2$. 1x TBE buffer for 1:30 hours. N: DNA ladder (100).

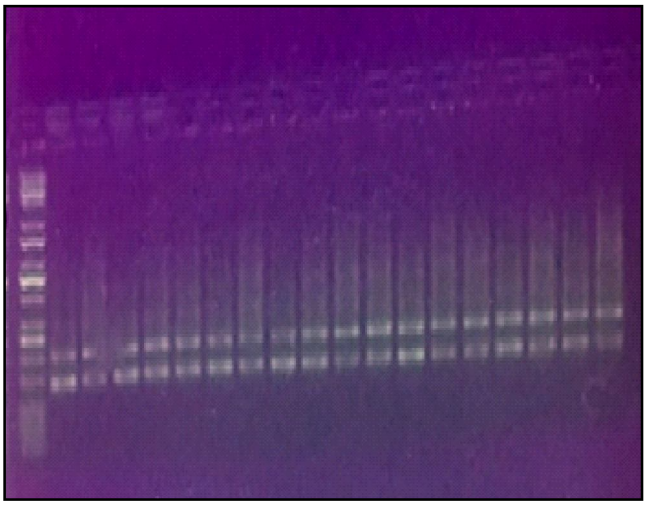

**Fig. 5:** PCR product of OP-V02 primer The product was electrophoresis on 2% agarose at 5 volt/cm$^2$. 1x TBE buffer for 1:30 hours. N: DNA ladder (100).

**Table 3:** Number of bands and RAPD markers per primer.

| Primer | Number of bands | Number of markers |
|---|---|---|
| OP-M06 (200-1000)bp | 111 | 7 |
| OP-R14 (350-1100)bp | 94 | 6 |
| OP-M20 (600-1800)bp | 81 | 5 |
| OP-I02 (200-1200)bp | 102 | 8 |
| OP-V02 (300-750)bp | 68 | 4 |
| OP-V19 (600-1800)bp | 66 | 4 |
| OP-K01 (400-1000)bp | 39 | 3 |
| OP-M14 (200-800)bp | 72 | 5 |
| Op-P04 (300-1300)bp | 84 | 6 |
| Op-P04 (400-1800)bp | 82 | 5 |

low number of bands value 4 bands while others primers had similarity, OP-R14 (P.2) gave difference to control cultivar (Muhood) by giving low value 2 bands, there were variations in OP-M20 primer (P.3), Baghdad 3, Alez and

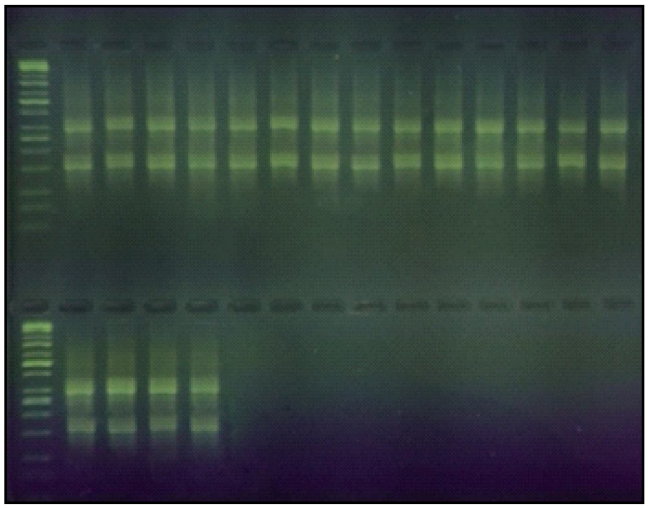

**Fig. 6:** PCR product of OP-V19 primer The product was electrophoresis on 2% agarose at 5 volt/cm$^2$. 1x TBE buffer for 1:30 hours. N: DNA ladder (100).

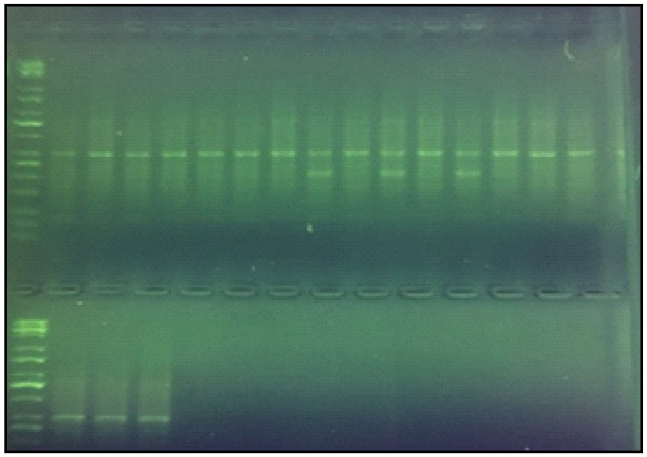

**Fig. 7:** PCR product of OP-K01primer The product was electrophoresis on 2% agarose at 5 volt/cm$^2$. 1x TBE buffer for 1:30 hours. N: DNA ladder (100).

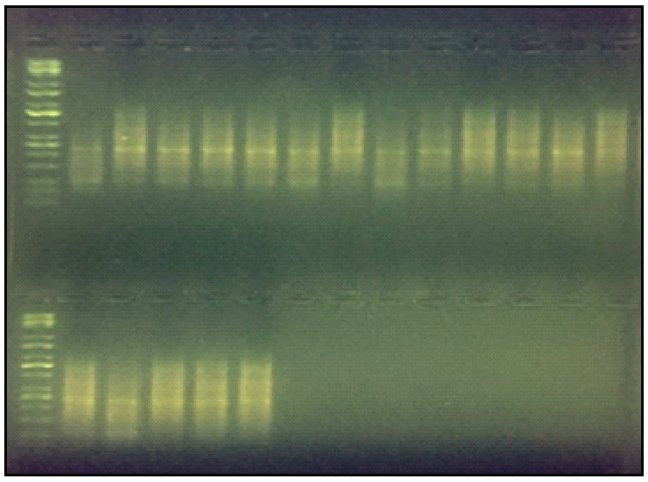

**Fig. 8:** PCR product of OP-M14 primer The product was electrophoresis on 2% agarose at 5 volt/cm$^2$. 1x TBE buffer for 1:30 hours. N: DNA ladder (100).

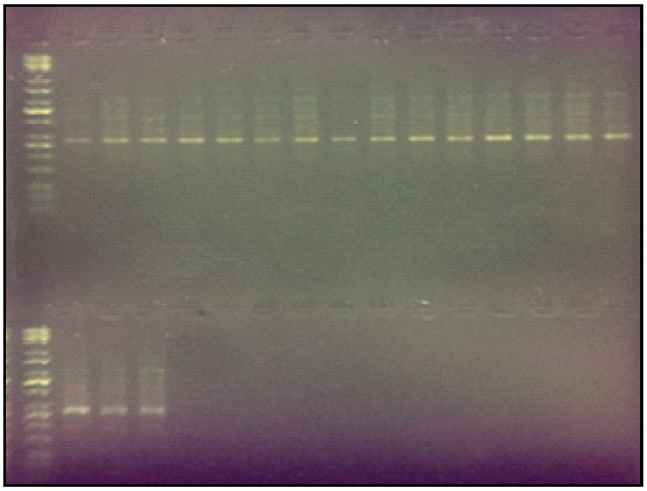

**Fig. 9:** PCR product of Op-P04primer The product was electrophoresis on 2% agarose at 5 volt/cm$^2$. 1x TBE buffer for 1:30 hours. N: DNA ladder (100).

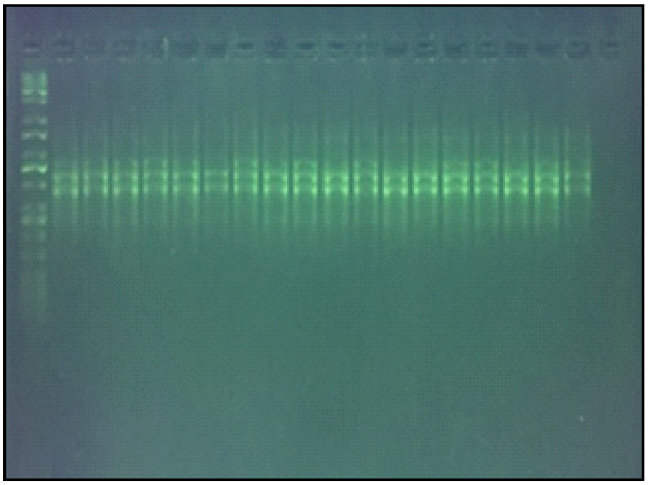

**Fig. 10:** PCR product of OP- H01 primer The product was electrophoresis on 2% agarose at 5 volt/cm$^2$. 1x TBE buffer for 1:30 hours. N: DNA ladder (100).

Iraq were similarity, Babi l113, furat and Buhooth 158 were also similarity, in OP-I02 primer (P.4), aboghreeb cultivar give higher number of bands while Latifia cultivar gave low number of bands, Babi l113 and Sham 6 were similarity, Baraka and Buhooth 10 were similarity too, OP-V02 primer (P.5) did not give clear variations but Buhooth 22 characterized by giving low bands, OP-V19 (P.6) did not give variations and characterized, Latifia gave low number of bands alone in OP-K01 primer (P.7), OP-M14 primer (P.8) gave similarity among Buhooth 22, Latifia and Buhooth 158, another similarity was among Furat, Baghdad1 Alez, Op-P04 primer (P.9) gave characterized to Babil 113 cultivar and similar Buhooth 22, there were similarity and differences between two groups of cultivars in OP-H 01 primer (P.10) table 4.

The control cultivar (Muhood) similar Ibaa 99 but it differed in primer 2 about all cultivars.

As shown in fig. 11, nine sets of cultivars were

**Table 4:** The interaction between primers and cultivars according to number of the bands.

| No. | Cultivar name | P. (1) | P.(2) | P.(3) | P.(4) | P.(5) | P.(6) | P.(7) | P.(8) | P.(9) | P.(10) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Babil 113 | 5 | 4 | 4 | 4 | 3 | 4 | 2 | 4 | 2 | 4 |
| 2 | Furat | 6 | 6 | 4 | 5 | 4 | 3 | 3 | 5 | 5 | 4 |
| 3 | Baghdad 3 | 7 | 6 | 3 | 6 | 3 | 4 | 2 | 4 | 5 | 4 |
| 4 | Buhooth 10 | 7 | 6 | 5 | 7 | 4 | 4 | 2 | 4 | 5 | 4 |
| 5 | Tamuz 2 | 7 | 6 | 5 | 6 | 4 | 4 | 2 | 4 | 5 | 4 |
| 6 | Rashid | 5 | 6 | 5 | 5 | 4 | 4 | 2 | 4 | 5 | 4 |
| 7 | Fares 1 | 7 | 6 | 5 | 5 | 4 | 4 | 3 | 4 | 5 | 5 |
| 8 | Buhooth 22 | 7 | 6 | 5 | 5 | 2 | 4 | 2 | 3 | 2 | 5 |
| 9 | Latifia | 7 | 6 | 5 | 3 | 4 | 3 | 1 | 3 | 5 | 5 |
| 10 | Baghdad1 | 4 | 6 | 5 | 5 | 4 | 4 | 3 | 5 | 5 | 5 |
| 11 | Sham6 | 7 | 6 | 5 | 4 | 4 | 4 | 2 | 4 | 5 | 5 |
| 12 | Ibaa 99 | 7 | 4 | 5 | 6 | 4 | 4 | 3 | 4 | 5 | 5 |
| 13 | Alez | 7 | 4 | 3 | 6 | 4 | 3 | 2 | 5 | 5 | 5 |
| 15 | Buhooth 158 | 5 | 5 | 4 | 6 | 4 | 3 | 2 | 3 | 5 | 5 |
| 16 | Iraq | 5 | 5 | 3 | 6 | 4 | 3 | 2 | 4 | 5 | 5 |
| 17 | Baraka | 5 | 5 | 5 | 7 | 4 | 3 | 2 | 4 | 5 | 4 |
| 18 | Aboghreeb | 6 | 5 | 5 | 8 | 4 | 3 | 2 | 4 | 5 | 4 |
| 14 | Muhood (C.) | 7 | 2 | 5 | 6 | 4 | 4 | 2 | 4 | 5 | 5 |
| Number of markers | | 7 | 6 | 5 | 8 | 4 | 4 | 3 | 5 | 6 | 5 |

obtained at the 70% agglomeration level (a critical value within a range from 1 to 9%) of the cluster analysis established from the matrix of Jaccard's identity which is based on the common occurrence and not on the common absence of a specific marker in a pair of cultivars.

Set 1 included two cultivars (Babil 113 and Buhooth 22), Set 2 made of a single cultivar (Furat), Set 3 constituted of 4 cultivars (Baghdad 3, Buhooth 10, Tamuz 2 and Rashid), Set 4 also constituted of 4 cultivars (Fares 1, Sham 6, Ibaa 99 and Baghda 1), Set 5 clustered in one (Latifia), Set 6 included one cultivar too (Alez), Set 7 found in cultivar (Buhooth 158), Set 8 constituted of 3 cultivars (Iraq, Baraka and Aboghreeb), Set 9 included Iranian cultivar of control (Muhood).

## Discussion

On the basis of the polymorphism generated by the 10 RAPD primers, 17 distinct genotypes were identified from the 18 wheat samples analyzed in the present study. 17 genotypes were specific to a single individual. One genotype was identified in two cultivars Buhooth 10 and Tamuz 2 (synonymy).

According to the dendrogramme and from 70% of similarity one could highlight 9 groups comprising 1 to 17

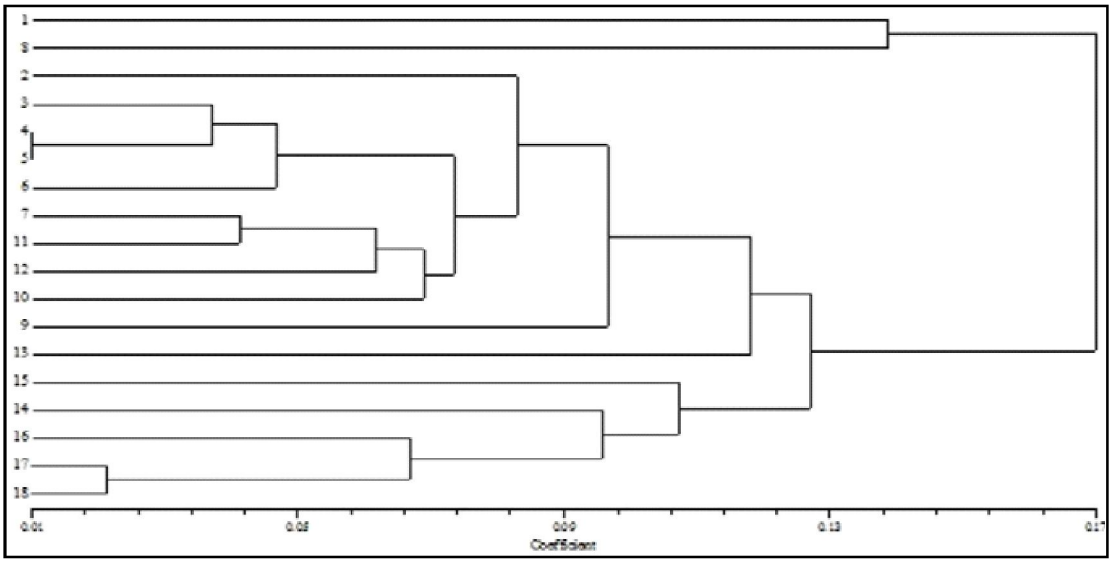

**Fig. 11.** genetic distance coefficient.

cultivars. The cultivars involved in different loci of genetic material, the amplification of monomorphic loci is depicting sharing of common blood among the genotypes (Asif *et al.,* 2005).

In the present experiment some specific RAPD bands were identified; thus reflecting the RAPDs application for the identification of wheat, which may correlate with morphological trait. It was observed that cultivars Buhooth 10 and Tamoz 2 which differed at 4 primer only , Bibi, *et al.,* (2009) and Manifesto *et al.,* (2001) found some specific RAPD marker while examining genetic diversity in spring wheat cultivars grown in the Yaqui Valley of Mexico and the Punjab of Pakistan.

Nei's and Lei's coefficient similarity matrix were calculated to estimate the genetic divergence and relatedness among wheat genotypes, genetically most similar cultivars were Faris 1, Baghdad 1, Sham 6 and Ibaa 99 (0.08) while most dissimilar cultivars were Buhooth 10 and Tamooz 2 against Babil 113, Moreover, these databases made breeders could share breeding material and the tendency to use genetically similar parents in breeding programs have led to a concern of lack of genetic diversity (Rehman *et al.,* 2002). The need to broaden the genetic base of germplasm is an area of concern in modern agriculture. Pyramiding crosses are suggested to increase the genetic diversity in the population (Jabbar, 2019 and Kose, 2017) and will be helpful in developing improved cultivars and breeds.

## Acknowledgements

Wahaj DNA Labs. were thanked for help us to take out these results.

## References

Ali, A.N., I. Ali, M. Adnan, N. Ullah and Z.A. Swati (2013). Morphological and genetic diversity of Pakistani wheat germplasm under drought stress. *International Journal of Advance in Res. and Tech.,* **2(5):** 186- 193.

AL-Salihy, A. and M.Kh. Jabbar (2017). *In Vitro*: Detection *TaSOS1* gene in four Iraq genotypes of Bread Wheat under different salt stress levels. *Iraqi J. of Biotech*, **16(4):** 69-78.

AL-Salihy, A., I. Hassan and M.Kh. Jabbar (2018). Study of *TaSOS4* gene and yield, chemical traits in some bread white genotypes under salinity conditions. *Iraqi J. of Agri. Sci.*, **49(4):** 586-591.

Asif, M., M. Rehman and Y. Zafer (2005). DNA fingerprinting studies of some wheat (*Triticum aestivum* L.) genotypes using random amplified polymorphic DNA (RAPD) analysis. *Pakistan J. of Bot.*, **37:** 271-277.

Balkan, A. (2018). Genetic variability , heritability and genetic advance for yield and quality traits in $M_{2-4}$ generations of bread wheat (*Triticum aestivum* L.) genotypes. *Turk. J. fields crops*, **23(2):** 173-179. http://dx.doi.org/10.17557/tjfc.485605.

Barakat, M.N., AA Al-Doss, K.A. Moustafa, E.L. Ahmed and A.A. Elshafei (2010). Morphological and molecularcharacterization of Saudi wheat genotypes under droughtstress. *J. of Food, Agri. and Environ.*, **8(1):** 220-228.

Bhutta, W.M., J. Akhtar, M. Ibrahim and A. Shahzad (2006). Genetic variation between Pakistani wheat (*Triticum aestivum* L.) genotypes as revealed by Random Amplified Polymorphic DNA (RAPD) markers. *South Africa J. of Bot*, **72(2):** 280-283. https://doi.org/10.1016/j.sajb.2005.09.005.

Bibi, S., M.U. Dahot, I.A. Khan, A. Khatri and M.H. Naqvi (2009). Study of genetic diversity in wheat (*Triticum aestivum* L.) using random amplified polymorphic DNA (RAPD) markers. *Pakistan J. of Bot.*, **41(3):** 1023-1027.

Cerasela, P., A. Lazar, P. Irina, I. Maria, V. Giancarla and C. Banu (2011). Somaclonal variation at the nucleotide sequence level revealed by RAPD and ISSR markers. *J. of horti., Forests. and Biotech.*, **15(4):** 119-123.

Guzman, M., L. Vilain and T. Rondon (2018). Increasing genetic diversity of cotton breeding programs in Venezuela. *Turk. J. field crops*, **23(2):** 151-158. http://dx.doi.org/10.17557/tjfc.484975.

Jabbar, M.K. (2019). Insertion red kidney beans in Iraqi food diversity program from production of cold tolerant lines by mutation technique. *Eur. J. of Biosci.*, **13(1):** 69-73.http://dx.doi.org/10.13140/RG.2.2.33088.58885.

Khan, N., I. Ahmad, M.T. Azhar (2017). Genetic variation in relative cell injury for breeding upland cotton under high temperature stress. *Turk. J. field crop*, **22(1):** 152-159. http://dx.doi.org/10.17557/tjfc.315544.

Kose, A. (2017). Gene action and combining ability in line X tester population of safflower (*Carthamus tinctorius* L.). *Turk. J. field crops*, **22(2):** 197-203. http://dx.doi.org/10.17557/tjfc.356216.

Manifesto, M.M., A.R. Schlatter, H.E. Hopp, E.Y. Suárez and J. Dubcovsky (2001). Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. *Crop Sci.*, **41:** 682-690.

Nei, M. and W. HLi (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the Nat. Acad of Sci.*, **76(10):** 5269-5273.

Noah, L.H. (2018). Selection Some of Drought Tolerance Genotypes in bread wheat (*Triticum aestivum* L.) Under In Vitro and In Vivo Conditions Based on Conventional and Molecular Approaches. M.A A thesis, institute of genetic engineering and biotechnology for higher studies –university of Baghdad, Iraq.

Rehman, M., D. Hussain and Y. Zafar (2002). Estimation of genetic divergence among elite cotton cultivars genotypes by DNA fingerprinting technology. *Crop Sci.*, **42:** 2137-2144.

Sneath, P.H. and A. Sokal (1973). Numerical taxonomy – the principals and practice of numerical classification. W.H. Freeman and Co., San Francisco.

Vierling, R.A. and H.T. Nguyen (1992). Use of RAPD markers to determine the genetic diversity of diploid, wheat genotypes. *Theory Appl. Genet. J.*, **84(7-8):** 835-8.http://dx.doi.org/10.1007/BF00227393.