Plant Archives Volume 20 No. 2, 2020 pp. 6533-6539 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ESTIMATION OF GENETIC VARIATION FOR SEVERAL SELECTED GENOTYPES OF BREAD WHEAT *TRITICUM AESTIVUM* L. DEPENDING ON RAPD TECHNIQUE

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Abstract

In order to determine the genetic variation among three Iraqi bread wheat *Triticum aestivum* L. Selected genotypes for salt tolerance, (2H and 4H and Furat cultivar (5H)) derived from breeding and improvement programs under conditions of salt stress, compared to the local salinity-sensitive cultivar (Iraq), molecular markers with Random Amplified Polymorphic DNA (RAPD) techniquewere used. The results showed that the ten primers used in this experiment gave clear bands with a total number of 144 bands produced from 54 binding sites, 19 of which, with a rate of 19.19%, were Monomorphic bands, while the other 35 bands, which accounted for 64.81%, were Polymorphic.sizes of bands ranged between 2400-100bp. The primer OPG03 showed the highest value of polymorphism percentage 100%, while the highest values of the discriminatory power percentage for primers and the percentage of primer efficiency produced by the primer UBC1 20%, 16.66%. The RAPD primers also showed a number of unique bands in some samples, reaching eleven bands, which can be considered a marker of that sample and thus the identification of the genetic fingerprint of that genotype. The results of the primers for RAPD technique were adopted in the analysis of the genetic distance between the genotypes and showed that the largest Genetic distance was between the cultivar Iraq (salt-sensitive) and the genotype 4H (salt-tolerant) was 0.82521, while the lowest Genetic distance was between the cultivar Furat (5H) and the genotype 2H (both tolerant to salinity) and reached 0.28421. The cluster analysis (dendrogram) showed that the studied genotypes were distributed into three main groups. The RAPD markers showed a high ability to distinguish between the studied genotypes and gave an idea of its potential to detect the genetic variation between the genotypes of wheat depending on the presence of the main Monomorphic and Polymorphic bands and their numbers.

Key words: Wheat *Triticum aestivum* L., RAPD, Genetic Variation, Genotypes.

Introduction

Wheat *Triticum aestivum* L. plant belongs to the Gramineae family, Genus Triticum (Al-Karkhi *et al.,* 2018). Wheat crop is one of the most important cultivated and produced cereal crops in the world (Elameen *et al.,* 2013). Out of a third of the world's population based on living mainly on it (Muhammad, 2015), the growth of wheat is affected by many biotic and abiotic stresses, including salinity, as a result of a series of physiological and biochemical changes that negatively affect plant growth and development, and this is even tually reflect on productivity (Munns and Tester, 2008; Asghari and Ahmadvand, 2018) Which posed challenges for wheat breeders to develop new genotypes of high yield performance and resistance to stress conditions (Motawei *et al.,* 2007), the study of the genetic distance between such genotypes and cultivars by morphological characteristics became insufficient because they are limited in number and affected by environmental conditions (Kumar *et al.,* 2016) Therefore, more accurate methods had been used . During the past few decades, the technique of DNA markers has gained great importance (Khan *et al.,* 2017) as it depends on the study of genotype itself without the impact of the environment on it, for that the molecular markers are of great importance in plant breeding and studying the genetic diversity and evolutionary relations between organisms, in addition to that it opened new horizons in the breeding and selection processes in terms of time, cost and

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accuracy in the results (Cifci and Yagdi, 2012). Many molecular techniques have been adopted in the study of the genetic diversity between wheat varieties spread around the world, including: RAPD technique (Kumar *et al.*, 2017; Ali *et al.*, 2013), ISSR technique (Majeed *et al.*, 2018) and SSR technique (Kumar *et al.*, 2016 and Al-Tamimi and Al-Janabi, 2019).

RAPD technique is one of the effective and affordable molecular markers in studying of genetic diversity between species and between taxa belonging to the same species and understanding the genetic relationship between them, and it was adopted to determine the genetic identity and to investigate the hybrids and genetic mapping (Hussain *et al.*, 2015; Al-Ghufaili and Al-Tamimi, 2018) and fingerprinting (Fadoul *et al.*, 2013).

The aim of this study is to detect the genetic variation and estimate the genetic distance by using the molecular markers of three genotypes (2H and 4H and Furat 5H)) derived from breeding programs for salinity tolerance and the local salt-sensitive cultivar (Iraq) using Random Amplified Polymorphic DNA (RAPD-PCR) technique and to give a genetic identity to each genotype.

Materials and methods

Cultivation of samples

The experiment was carried out at the Biotechnology Research Center , Al-Nahrain University, Baghdad - Iraq. In this experiment, two genotypes of bread wheat *T. aestivum* (2H and 4H) and Furat cultivar (5H) derived from breeding and improvement programs for salinity tolerance and the local salt-sensitive cultivar (Iraq) were used.

Ten wheat seeds were sown in each pot and for each genotype and cultivars, at the end of the booting stage a number of young plant leaves were taken form each genotype for DNA extraction.

DNA extraction

Genomic DNA was isolated from the young wheat leaves using the method (CTAB Cetyl Trimethyl Ammonium Bromide) using Hamorabi Geno Tech Kit (from the Institute of Genetic Engineering / University of Baghdad) . DNA concentration and purity was measured by the Nano drop and DNA quality was estimated by electrophoresis at a 1% agarose gel and a Red Safe dye and imaging it under a UV light.

Random Amplified Polymorphic DNA (RAPD-PCR) analysis

In this experiment, ten random primers were used table 1 supplied by (Bioneer-Korea) Company Lyophilized was dissolved using Distilled Water to obtain the final concentration of 10 pmol / ml. and the Master Mix was used by (AccuPower® PCR PreMix) also supplied by Bioneer-Korea.

PCR amplification was performed by adding the substances shown in table 2 to the Master Mix tube (the final reaction volume for each tube is 20µl). The amplifications were carried out in Lab net-USA thermocycler, the program shown in table 3.

PCR products were separated on 1% agarose gels and stained with Red Safe nucleic acid staining solution.

Data Analysis

1. RAPD bands were scored by placing 1 for presence and 0 for absence and accordingly genetic variation and genetic distance were calculated by the unweighted pair group method using arithmetic average (UPGMA) and the dendrogram was drawn using Past *ver.* 1.91 software (Hammer *et al.*, 2001).

2. Polymorphism % = (Np/Nt) x 100 (Al-Hadeithi, 2012)

Np = number of polymorphic bands of the primer

Nt = total number of bands of the same primer

Table 1: Primers and its sequences used in the experiment.

No.	Primer	Sequence
1	UBC1	CCT GGG CTT C
2	UBC16	GGT GGC GGG A
3	UBC76	GAG CAC CAG T
4	Op-B01	GTT TCG CTC C
5	Op-B11	GTA GAC CCG T
6	B-14	ACCCCCGAAG
7	AD-08	GGCAGGCAAG
8	OPG 03	GAGCCCTCCA
9	OPH 04	GGAAGTCGCC
10	OPF 09	CCAAGCTTCC

Table 2: Components of RAPD-PCR reaction.

Material	Final concentration	Volume for single tube
Deionised D.W	-----	µl16
Primer	10 pmol/µl	µl2
DNA template	100 ng	µl2

Table 3: (RAPD-PCR) program.

Step	Temperature	Time	No. of cycles
Initial Denaturation	94°C	4 min	1
Denaturation	92°C	1 min	36
Annealing	35°C	1 min	
Extension	72°C	2 min	
Final Extension	72°C	5 min	1

3. Discriminatory power percentage = (number of polymorphic bands in a primer / number of polymorphic bands in all primers) x 100 (Al-Judy and Majeed, 2013).

4. Primer efficiency percentage = (number of total bands in aprimer / number of total bands in all primers) x 100.

Results and discussion

PCR results showed a clear difference in number of bands according to the primer used. The ten primers combined gave 144 bands produced from 54 binding sites, of which 19 bands were with a rate of 35.19% Monomorphic, as they appear in all genotypes, while polymorphic bands was 35, which constituted 64.81%, which determine the genetic link between the studied genotypes. The primer UBC1 gave the highest number of polymorphic bands which was 7 bands, while the primer AD-08 produced the lowest number of polymorphic bands, which was one band, it is also noted that molecular weights of bands ranged between 2400-100 bp. polymorphism percentage varied in each primer,

Table 4: The samples and primers that showed unique bands for these samples with the molecular weight of these bands.

No.	Sample	Primer	Molecular weight of unique bands/bp
1	4H	OPH04	150
2	4H	OPH04	600
3	4H	OPH04	750
4	Iraq	OP-B01	600
5	Furat	UBC76	300
6	Furat	OPF09	300
7	Furat	UBC1	300
8	Furat	UBC1	600
9	Furat	UBC1	900
10	4H	UBC1	2000
11	Furat	UBC16	250

Table 5: Product of random primers used in this study, polymorphic bands,polymorphism percentage, percentage of Discriminatory power, Primer efficiency percentage.

No.	Primer	Main bands	Polymorphic bands	Polymorphism %	Discriminatory power %	Primer efficiency %
1	OPHO4	8	6	75	17.14	14.81
2	OP-B01	6	2	33.33	5.71	11.11
3	UBC76	5	2	40	5.7	9.25
4	B-14	5	3	60	8.57	9.25
5	OPF09	8	6	75	17.14	14.81
6	OP-B11	4	2	50	5.71	7.40
7	UBC1	9	7	77.77	20	16.66
8	UBC16	4	3	75	8.57	7.40
9	AD-08	2	1	50	2.85	3.70
10	OPG03	3	3	100	8.57	5.55
Sun		54	35	----	----	----
Average		----	64.81	63.61	9.996	9.994

Table 6: Genetic Distancematrix for wheat cultivars and Genotypes.

	Furat	2H	4H	Iraq
Furat	0			
2H	0.28421	0		2H
4H	0.33478	0.39565	0	4H
Iraq	0.78421	0.72727	0.82521	0

Highest genetic distance lowest genetic distance

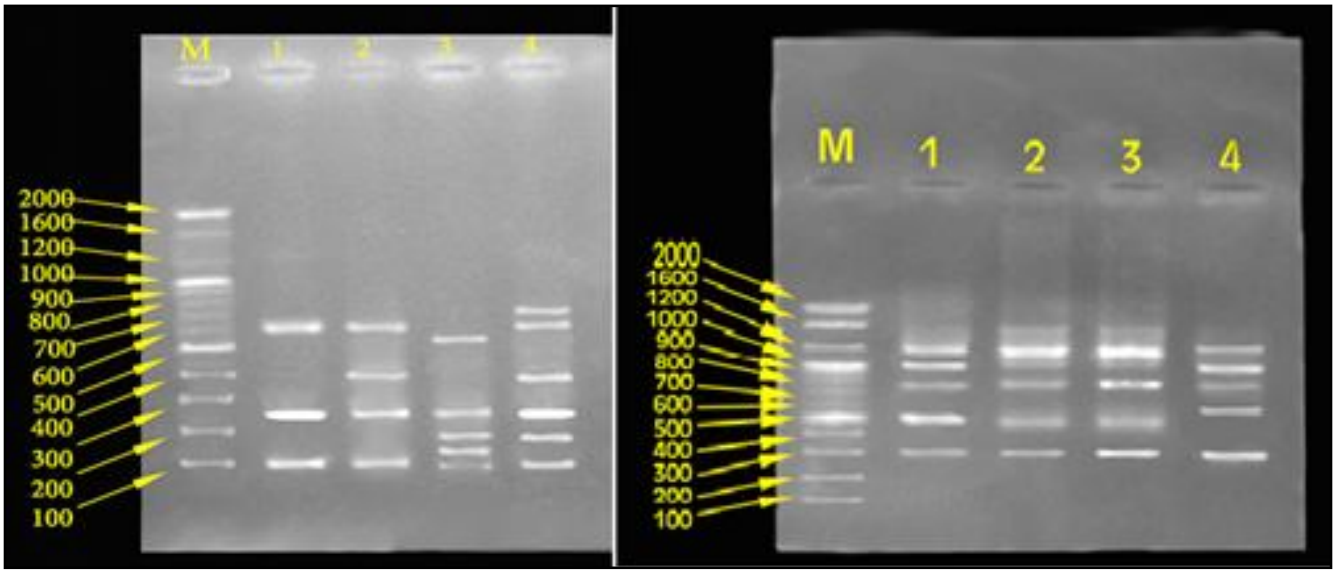

Fig. 1: RAPD-PCR product of OPH04, OP-B01 primers on 1% agarose gel (90 minutes, 5V/cm, 1X TBE) stained with Red Safe. dye, M= marker DNA Ladder 100 bp, 1-Furat, 2- 2H, 3- 4H, 4- Iraq.

the primer OPG03 gave the highest percentage of polymorphism 100% followed by UBC1 77.77%, that refers to the high ability of some primers to detect variations between genotypes and cultivars, as they have been able to identify complementary sequences in their DNA, table 4. The difference at the sites where bands are distributed may be due to the difference in the genetic base from which those genotypes came from (Al-Timime, 2019). RAPD primers showed eleven unique bands in some samples that can be an indicator of these samples and thus determine the genetic fingerprint of those genetic structures ,six of which distinguished Furat cultivar and 4 bands distinguished the genotype 4H, table 5, These results are consistent with (Elameen *et al.*, 2013; Mueen and Jabbar, 2019) in adopting these bands as markers for these genotypes. Discriminatory power of the primers showed a good ability to distinguish the genotypes of wheat, the highest value was 20% for the UBC1, while the highest value for the percentage of primers efficiency was 16.66 also for the UBC1. These values gave a clue about RAPD ability to detect the genetic distance between wheat genotypes (tolerant to salinity and sensitive) depending on the presence of monomorphic and polymorphic bands. In general results have shown that there is a large percentage of genetic differences, which confirms the effectiveness of this technique in distinguishing between wheat genotypes grown in Iraq, even when there is a low degree of genetic differences (wheat samples resulting from the breeding and improvement program), this technique was able to

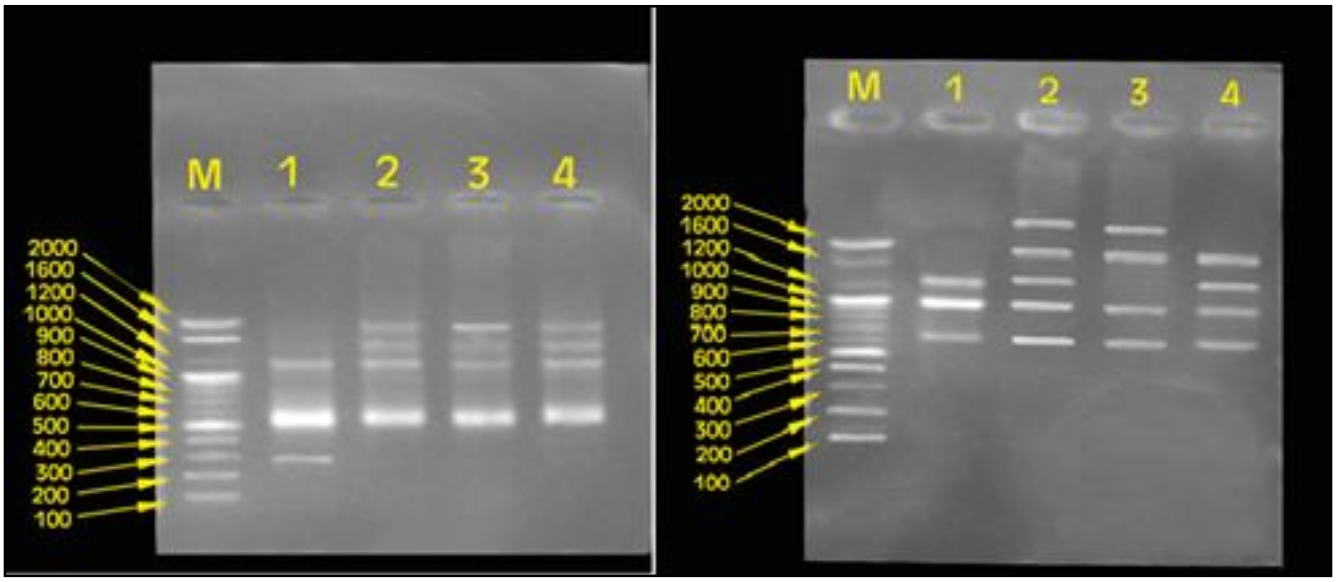

Fig. 2: RAPD-PCR product of UBC76, B-14 primers on 1% agarose gel (90 minutes, 5v/cm, 1X TBE) stained with Red Safe dye, M = marker DNA Ladder 100 bp, 1-Furat, 2-2H, 3-4H, 4-Iraq.

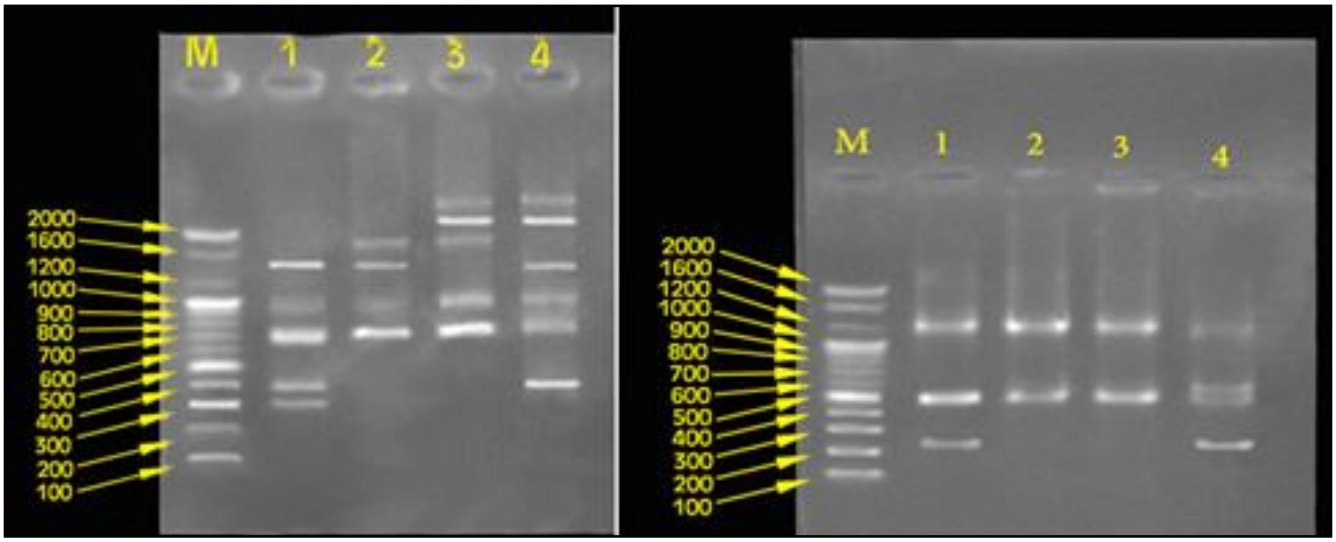

Fig. 3: RAPD-PCR product of OPF09, OP-B11 primers on 1% agarose gel (90 minutes, 5v/cm, 1X TBE) stained with Red Safe dye, M = marker DNA Ladder 100 bp, 1-Furat, 2-2H, 3-4H, 4-Iraq.

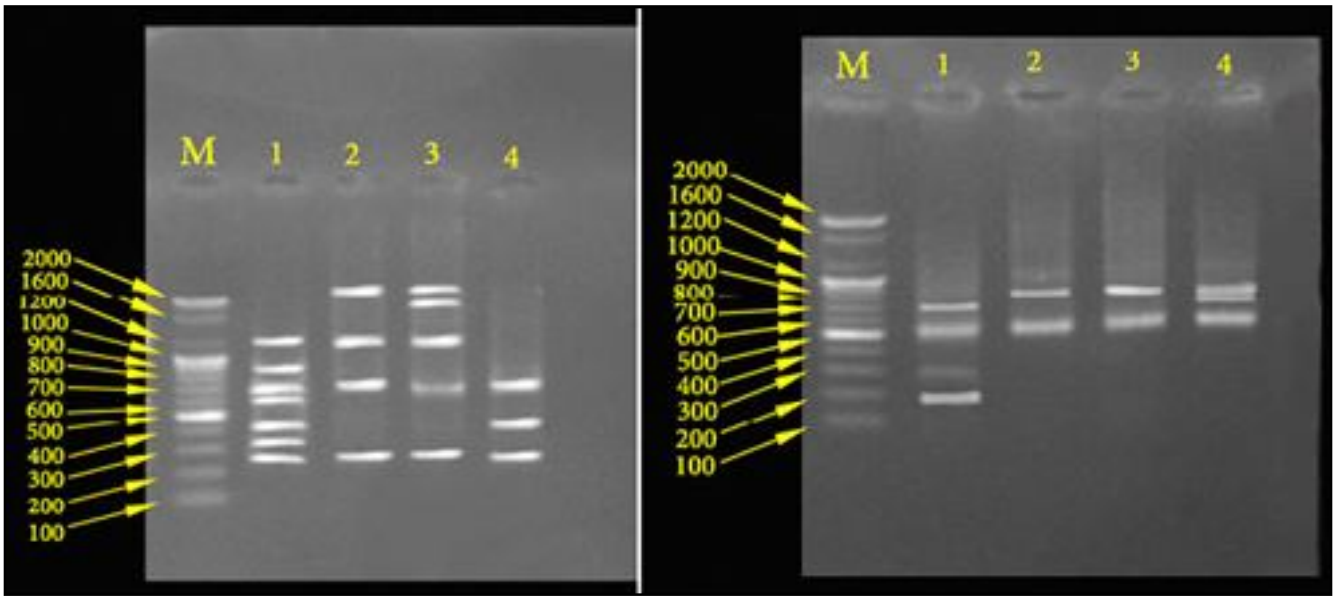

Fig. 4: RAPD-PCR product of UBC1, UBC16 primers on agarose gel (90 minutes, 5v/cm, 1X TBE) stained with Red Safe dye, M = marker DNA Ladder 100 bp, 1-Furat, 2-2H, 3-4H, 4-Iraq.

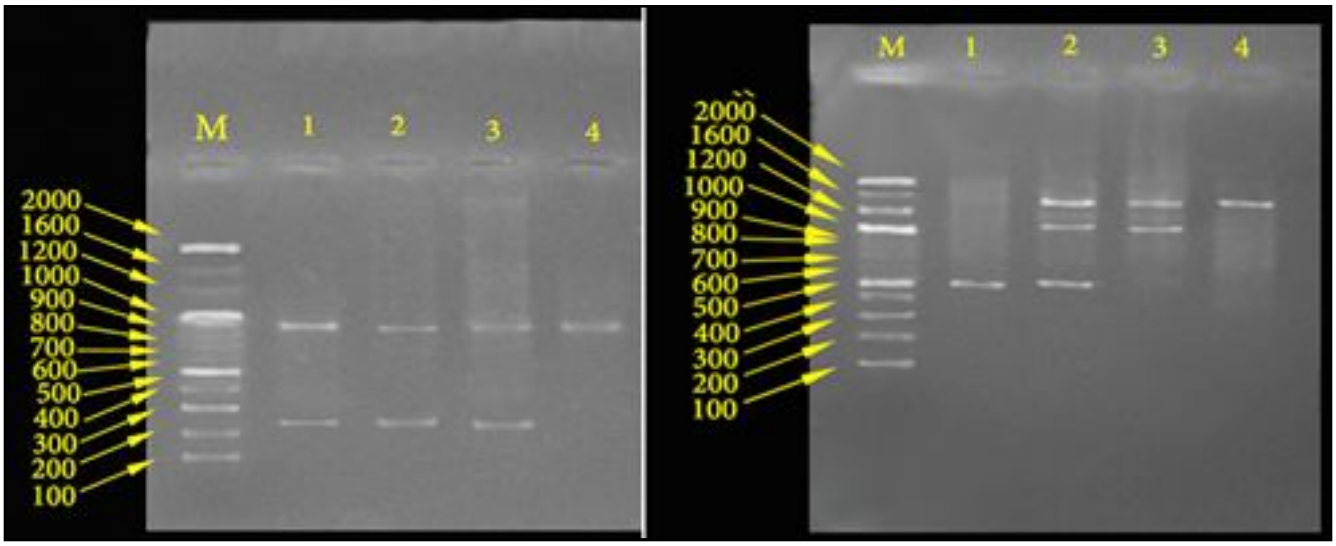

Fig. 5: RAPD-PCR product of AD-08, OPG03 primers on agarose gel (90 minutes, 5v/cm, 1X TBE) stained with Red Safe dye, M = marker DNA Ladder 100 bp, 1-Furat, 2-2H, 3-4H, 4-Iraq.

distinguish the genetic structures which differs in one band and these results are consistent with many studies (Al-Karkhi, 2018; Al-Tamemy, 2018; Al-Timime, 2019; Alsaady, 2015; Majeed *et al*., 2018; Al-Tamimi and Al-Janabi, 2019) which were characterized by the presence of high levels of genetic variation. It becomes clear to us the importance of using random primers targeting many regions of the genome, this helps in showing the difference between the genotypes studied according to the sequence of the primer used and complementary sequence in the genotypes. (Al-Karkhi, 2018; Al-Ghufaili and Al-TAmimi, 2018).

Genetic distance, table 6 shows that the lowest genetic distance 0.28421 was between Furat (5H) and the genotype 2H (both salt-tolerant), while the highest genetic distance was between Iraq (salt-sensitive cultivar) and 4H genotype (salt-tolerant) was 0.82521 and this is consistent with Al-Karkhi, (2018), where the highest genetic distance between the studied genotypes was 0.791, and the results also showed that 2H genotype was more genetically closely related to the 4H genotype compared to Iraq cultivar because they are sharing in a small number of bands due to differences in the nucleotide sequence in the genome of this genotype and the local cultivar.

Dendrogram was obtained based on the results of RAPD technique by using Dice coefficient of genetic similarity. From the Fig. 6 it is clear that the samples were included in the

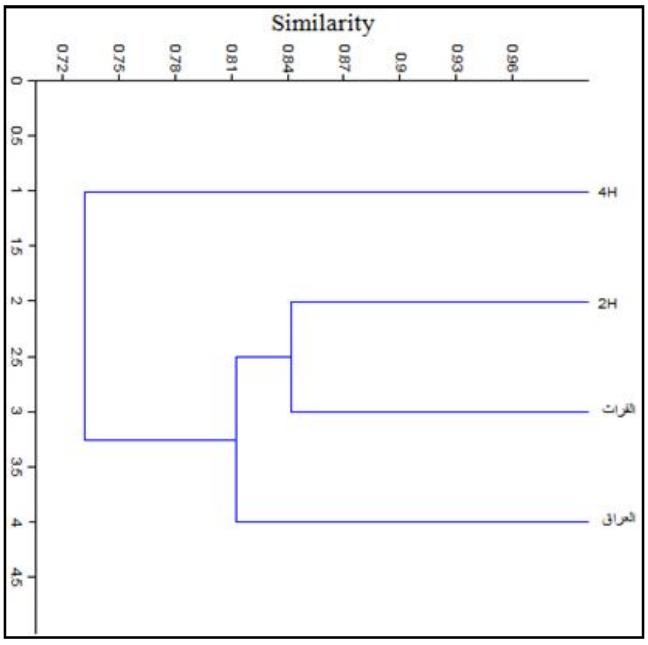

Fig. 6: Dendrogram based on the results of RAPD technique for the genotypes and cultivar studied according to Dice coefficient of genetic similarity.

cluster analysis in three main groups: The first group: included only 4H genotype . The second group: included both 2H and Furat. The third group: included Iraq cultivar only, this results consistent with Alsaady, (2015) in isolating the salt-sensitive cultivar in a separate group in the dendrogram ,results also indicated that Furat cultivar was more closely related to 2H genotype and more distant with the Iraq cultivar.

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