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Genetic Diversity Of Some Barley Cultivars Using Rapd Dna Analysis

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Abstract

The current study aimed to assess the genetic diversity of six cultivars of barley (Samir, Buraq, Amal, Shahah, Alhadr, IPA 99) using the PCR-based Random Polymorphism (RAPD) technique for ten primers, which produced 64 bands , including 26 polymorphism bands. With a genetic variance of (40%).Analysis of the genetic distance of the cultivars showed that the largest genetic distance was between the two cultivars Shahah and Buraq (0.185). While the lowest genetic distance was between the Alhadr and Amal cultivars (0.027), and the cultivars were classified into two main groups, the first group included Buraq and the second group for the rest of the cultivars. The RAPD parameters were highly efficient in diagnosing the genetic link between cultivars, It will also serve as an indicator for plant breeders, especially in future studies of this crop.

Keywords: barley, genetic divergence, RAPD.

Introduction

Barley (Hordeum SPP.) is an important global and locally important winter feed grain crop. It is one of the ancient field crops known before wheat, and is cultivated all over the world, due to its tolerance of harsh environmental conditions as well as its low nutritional requirements. It represents an alternative to the wheat crop in food for many countries of the world, especially in areas with a low average of rain. It represents the fourth place in the world in terms of cultivated areas and grain production, and the cultivated area of the barley crop in all of Iraq for the seasons 2018-2019 and 2019-2020 was 930313 and 13212 hectares, respectively, according to the statistics and reports of the Central Statistics Department.

It is of more nutritional value than most other crops. It is used to make bread after mixing it with certain proportions of bread flour and a substitute for rice after removing the peel. In addition to its industrial uses, it is used medically as a laxative and a fever reducer, and in the manufacture of malt, (Gani 2011).

The territory of Iraq still suffers from many problems that have led to a decrease in its production of yield per unit area compared to international rates. There are problems related to the cultivar and others related to uncontrolled factors such as environmental factors, which can interfere with the cultivars or alone. Continuing to cultivate the old cultivars has also led to a decrease in the production of this crop. The DNA indicators are of

great importance from the biochemical and morphological indicators because they do not affect the environment and depend on the genome sequence or genetic material.

It is possible to detect differences in the genetic material carried by an individual, and in recent times the use of these techniques has increased to characterize and identify important genetic materials in addition to that to know the genetic distance between parents. Amplified Polymorphic Dna), which is characterized by ease, low cost, and dependence on DNA sequencing crystallization technique (Abdul Hamid and Bektas, 2014).Among the previous researchers who dealt with the study of genetic variations between genotypes and the distance between them are Rizkalla et al. (2012), al-Karkhi et al. (2018) and Anis and al-Dulaimi (2020).Based on the importance of the foregoing, the current study aimed to characterize some genotypes of some barley cultivars using RAPD molecular analysis.

Materials and methods

field experiment

The field experiment was carried out in the winter season of the year (2020-2021) in the fields of a farmer in Al-Dhuluiya District, Salah Al-Din province. It was planted on 10/20/2020. Where a factorial experiment was conducted using a randomized complete block design (RCBD).Where the experiment land was prepared by tillage it twice and in an orthogonal manner, whereby a disc plow was used for this purpose, and the land was smoothed, leveled . The experimental land was divided into three replicates, each replicates containing (18) experiment units, and each experimental unit included (5) lines for cultivation, the length of one line (3 m) and the distance between each line and another (0.2 m) with a quantity of seed (8 g).

Table (1) the numbers, symbols, and names of the genotypes used in the study

No.	genotypes	Ratios	References
1	Samir	Irradiation of a local black hybrid with two rows * Arivan six rows F3 gamma rays dose 20 kilo rads, sifted from many isolates in 1994.	Atomic Energy Organization
2	Buraq		Atomic Energy Organization
3	Amal	Irradiation of the Nomar cultivars with a gamma ray of 20 kilorads in 1994.	Atomic Energy Organization
4	Shahah	Erivan fast neutron irradiation in Pakistan in 1994.	Atomic Energy Organization
5	Alhadr	Irradiation of the local black hybrid * Arivan in the third generation F3 with gamma rays of 20 km rad	Atomic Energy Organization
6	IPA 99		IPA Center for Agricultural Research

laboratory experiment

The process of extracting DNA from young leaves of barley plants in the seedling stage of the cultivars used in this study was conducted using the protocol prepared by the company that produced the kit produced by Zamo Research USA. Ten random prefixes were used, the details of which are shown in Table (2), which were provided by (Integrated DNA Technologies company, Canada).

Table (2) The random primers used in the study and their sequences

	Primer	Sequence
1	OP-I02	GGAGGAGAGG
2	OP-R06	GTCTACGGCA
3	OP-V02	AGTCACTCCC
4	OP-V09	TGTACCCGTC
5	OP-R12	ACAGGTGCGT
6	OP-M14	AGGGTCGTTC
7	OP-T20	GACCAATGCC
8	OP-M20	AGGTCTTGGG
9	OP-V19	GGGTGTGCAG
10	OP-N16	AAGCGACCTG

The Primers were dried and dissolved with ddH2O (Deionized distilled water) for each primer and the solution was mixed well to get a final concentration of 100 pcmol.ml-1 as the ready solution, and stored at -20°C until ignition. As for the solution used daily, it was prepared by withdrawing 10 microliters of the prepared solution and adding 90 microliters of ddH2O deionized water to make the final volume 100 ml until the reaction is conducted. The polymerase chain reaction (PCR) was conducted using a Maxime PCR Pre Mix Kit (i-Taq) supplied by iNt RoN, DNTPs, Reaction buffer (10X) and Gel loading buffer in volumes (5U/µl, 2.5mM, 1X and 1X), respectively. A PCR reaction was conducted with a volume of 25 µl containing the following components Taq PCR Pre Mix, primer, DNA and ddHO, in volumes (5 µl, 10 picols/µl (2 µl), 1.5 µl, and 16.5) µl, respectively. Where the reaction mixture was prepared according to the following: The reaction components were mixed using a micro pipeti and It was placed in a centrifuge, and 11 sterile 0.5 ml tubes were prepared, labeled according to the samples to be studied, and 23 µl of the mixture (Table 3) was added to each tube. And adding 1.5 µl of DNA to each sample in the tube designated for it, leaving tube 11 for Negative DNA, taking into account the distribution of the above mixture on the tubes, it must be before transferring the DNA to avoid contamination. Then transfer the tubes to the centrifuge for several seconds in order to collect the droplets adhered to the tubes and lower them to the bottom of the tube to maintain the final volume of the reaction mixture 25 µl. Finally, we put the tubes of the above mixture into a thermopolymerization PCR device

according to Rabbani et al. (2008).The solutions used are: TBE solution prepared with X1 strength, loading buffer, and red safe dye, which is a new and safe nuclear acid substance, an alternative to the traditional dye ethidium bromide (EtBr) used to detect DNA in Agarose gel. This pigment gives off green radiation when bound to DNA or RNA. Agarose gel was prepared at a concentration of 1.5% according to Sambrook et al. (1989) by dissolving 1.5 g of Agarose in 100 ml of 1X TBE solution. We use a 500 ml beaker and add 450 ml of distilled water and 50 ml of 10X TBE solution. Shake the beaker with its contents. Well by hand, then heated in a Microwave oven at a temperature of 100°C until it is completely melted and becomes a clear liquid free of bubbles, then cools below a temperature of 45-50°C, then 2 microliters of the safe red dye is added to the beaker containing the Agarose.

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$$\text{Genetic Distance}=1-\left(\frac{2*N_{xy}}{N_y+N_x}\right)$$

So that: GD represents the genetic distance , Nxy represents the number of bands shared between the two models x and y that represent two samples, Nx represents the number of total bands in the sample x and Ny represents the number of total bands in the sample y. Drawing a cluster analysis chart according to the UPGMA method (Sneath and Sokal, 1973).Using the ready program NTSYS -PC (Numerical Taxonomy System) to obtain the kinship tree or genetic distance , Thus, obtaining the aggregate analysis scheme that shows the proximal and distal genotypes of all studied samples. The polymorphism or the ratio of varying beams for each initiator was calculated according to the following equation

Percentage (%)polymorphism per primer= (number of dissimilar bands in primer / total number of primer bands) ˟100

The percentage of the discriminatory ability of each primer was calculated according to the following equation:

The discriminatory ability of each primer % = (Number of variant bands of the primer/ Number of variant bands of all primer)˟ 100

primer efficiency % = (total number of primer bands / total number of bands of all primer) ˟ 100

Results and discussion

In order to determine the genetic distance between the studied cultivars and evaluate their effectiveness in showing the differences between them, this test was conducted based on the RAPD technique, and the necessary molecular analyzes were conducted for them. The results of RAPD-PCR shown in Table (4) gave that all ten primers gave 64 clear bands, These primers differed in showing the polymorphic bands that depend on determining genetic variations between the same cultivars. It reached 26 binding sites, and also differed in their molecular weights resulting on an Agarose gel. Therefore, the percentage of genetic variance is 40% and the percentage of similarity is 60%, and the primers OP-R06 and OP-V09 gave the highest number of total bands that reached 10 link bands for each, while the primers OP-I02 gave the least number of total bands which amounted to only two bands. The primers OP-R06 gave the highest number of different bands , which amounted to seven different bands , because it has different link sites, the highest formal polymorphism amounted to 70%, and the highest efficiency rate reached (15.62%) with a discriminatory ability of (86.92%).The primers OP-I02, OP-M14, OP-M2O and OP-V19 gave the least number of polymorphic bands (one for each), The ten primers used in this study were characterized by a molecular weight ranging between 75-2000 bp, depending on the number of bands resulting from the association of the primers with the cultivars used in this study, the molecular sizes of the bands , and the polymorphism value of the primers .These results are consistent with those of Cific and Yadgi (2012), Al-Ani (2013), AL-Hamadiny et al. (2014), Al-Karkhi (2018), Anis and Al-Dulaimi (2020) and Al-Majma'i and Anis (2020).

Table (4) The primers outputs from the total and polymorphic bands with their efficiency ratios and discriminatory ability for the studied cultivars.

primer efficiency %	the discriminatory ability%	Polymorphism %	total number of polymorphic bands	total number of bands	primers	No.
3.125	3.846	50	1	2	OP-I02	1
15.625	26.923	70	7	10	OP-R06	2
9.375	7.692	33.33	2	6	OP-V02	3
15.625	11.538	30	3	10	OP-V09	4
12.5	19.230	62.5	5	8	OP-R12	5
14.0625	3.846	11.11	1	9	OP-M14	6
12.5	11.538	37.5	3	8	OP-T20	7
6.25	3.846	25	1	4	OP-M20	8

4.6875	**3.846**	**33.33**	**1**	**3**	**OP-V19**	**9**
6.25	**7.692**	**50**	**2**	**4**	**OP-N16**	**10**
			26	**64**		

1- primers OP-I02

1- primer OP-I02

This primer OP-I02 achieved ten duplications on agarose gel with an efficiency of 3.12% with binding at two allelic sites, one of which is identical in all cultivars as a main bundle at the molecular site 45bp and this is according to Table (5)It achieved a clear difference at one site at a molecular weight of 500bp, with a percentage of 50% and a discriminatory ability (3.8%).

Table (5) The individual polymorphic and nonpolymorphic bands with their molecular weights for the primer OP-I02

molecular weights	1	2	3	4	5	6	
500	0	0	1	1	1	1	
450	1	1	1	1	1	1	OP-I02

Absence of band : 0 band appearance: 1

2 - primer OP-R06

It is noted that the number of sites produced by this primer is ten sites, of which 7 are different sites, the percentage of variation is 70%, and their molecular sizes ranged from (175-1000) pb.Three bands were unique to the cultivar Samir at molecular sizes (275, 225 and 175) pb, with a discriminatory ability(26.92%) and an efficiency of 15.62% as shown in Table (6) and Figure (1).

Table (6) The individual polymorphic and non-polymorphic bands with their molecular weights for the primer OP-R06.

molecular weights	**1**	**2**	**3**	**4**	**5**	**6**	**OP- R06**
1000	**1**	**1**	**1**	**1**	**1**	**1**	
900	**1**	**1**	**0**	**0**	**0**	**0**	
700	**0**	**0**	**1**	**1**	**1**	**1**	
600	**1**	**1**	**1**	**1**	**1**	**1**	
500	**0**	**0**	**1**	**1**	**1**	**1**	
375	**1**	**1**	**1**	**1**	**1**	**1**	

300	**1**	**1**	**0**	**0**	**0**	**0**	
275	**0**	**1**	**0**	**0**	**0**	**0**	
225	**0**	**1**	**0**	**0**	**0**	**0**	
175	**0**	**1**	**0**	**0**	**0**	**0**	

Absence of band : 0 band appearance: 1

3 - primer OP-V02

This primer contributed to the doubling of the DNA of the cultivars, and the image of the migration of the PCR product on the agarose gel (Table 7) showed the appearance of 32 binding sites for all the cultivars in six alleles sites, with variation in two sites, where the percentage of differentiated bands was 33.33%,The efficiency of the initiator was relatively good, reaching 9.37%, and this primer was able to identify the complementary sequences of the genome of the cultivars and gave a clear difference in the DNA of the site and the molecular weights that ranged between 450 and 1000 bp,The results also showed that the value of the discriminant ability was 7.69%. The appearance of these sequences in a particular genome and their absence in another is a measure of genetic divergence between the two genomes (Al-Karkhi, 2018).

Table (7) The individual polymorphic and nonpolymorphic bands with their molecular weights for the primer OP-V02.

molecular weights	1	2	3	4	5	6	
1000	1	0	1	1	1	1	OP-V02
900	1	0	1	0	1	0	
650	1	1	1	1	1	1	
550	1	1	1	1	1	1	
500	1	1	1	1	1	1	
450	1	1	1	1	1	1	

Absence of band : 0 band appearance: 1

4 - primer OP-V09

This primer produced 51 total bands for all studied cultivars with an efficiency of 15.62% by linking the primer to ten alleles sites.Six bands of them are the same in all cultivars at the molecular weight (1100, 560, 600, 400, 375, 200 bp). As shown in Table (8), the discriminatory ability was 11.53% and the polymorphism for primer was 30%, while its efficiency was 15.62%.

Table (8) The individual polymorphic and nonpolymorphic bands with their molecular weights for the primer OP-V09

molecular weights	1	2	3	4	5	6	
2000	1	1	1	1	1	0	
1100	1	1	1	1	1	1	
1000	0	0	1	1	0	1	
700	1	1	1	1	1	0	
650	1	1	1	1	1	1	
600	1	1	1	1	1	1	
400	1	1	1	1	1	1	
375	1	1	1	1	1	1	
300	1	1	0	0	0	0	
200	1	1	1	1	1	1	OP-V09

Absence of band : 0 band appearance: 1

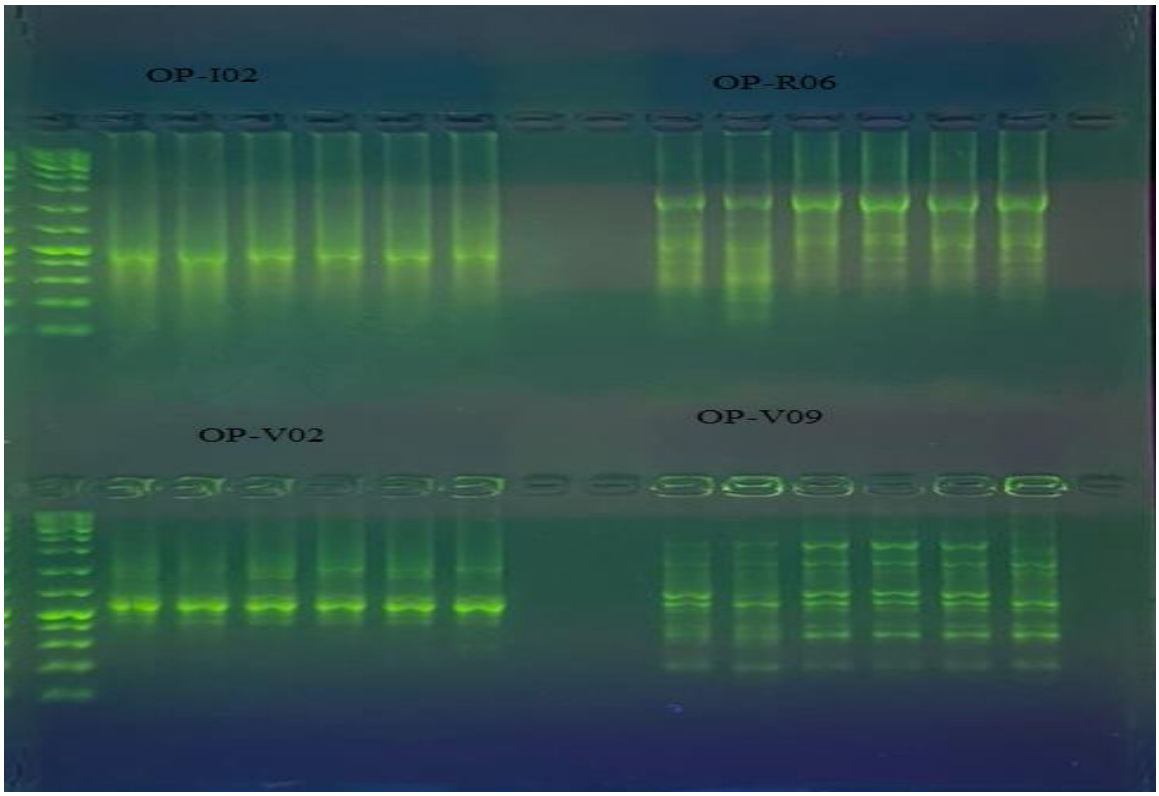

Figure (1) primer results (OP-I02, OP-R06, OP-V02, OP-V09) on 1% agarose gel

5 - primer OP-R12

The use of this primer OP-R12 resulted in a number of total bands amounting to 39 total bands, where they were associated in 8 alleles sites, 5 of which were of varying appearance between species and located between molecular weights (1750, 700, 325, 250 and 200 bp) at the same time representing the percentage of bands The high disparity amounted to five, and the formal polymorphism reached 62.5%.The discriminatory ability was 19.23% with an efficiency of 12.5%, and it is noted from Table (9) that this primer gave compatibility between the primer sequence and the genome DNA sequences in all cultivars in bands with molecular weights (450, 350, 300 pb).And that this discrepancy between the cultivars in the sites in which the diploid is distributed may be due to the difference in the genetic base from which these cultivars descend (Zakaria, 2011).

Table (9) The individual polymorphic and nonpolymorphic bands with their molecular weights for the primer OP-R12

molecular weights	1	2	3	4	5	6	
1750	1	0	1	1	1	1	
700	1	0	1	1	1	1	
450	1	1	1	1	1	1	
350	1	1	1	1	1	1	
325	0	1	1	0	0	0	
300	1	1	1	1	1	1	
250	1	1	1	0	1	0	
200	0	1	1	1	1	1	OP-R12

Absence of band : 0 band appearance: 1

6 - primer OP-M14

This primer OP-M14 was associated with 9 sites, eight of which were identical in all cultivars as major bands at molecular weights between 200 and 900 bp, where this primer was able to identify the complementary sequences in the genome DNA of the studied cultivars. The polymorphism amounted to 11.11%, with a discriminatory ability of 3.84%, with an efficiency of 14.06%.While the Shueae cultivars gave an absent band at a molecular size of 100bp according to Table (10).

Table (9) The individual polymorphic and nonpolymorphic bands with their molecular weights for the primer OP-M14

molecular weights	1	2	3	4	5	6	
900	1	1	1	1	1	1	
700	1	1	1	1	1	1	
600	1	1	1	1	1	1	
525	1	1	1	1	1	1	
500	1	1	1	1	1	1	
400	1	1	1	1	1	1	
300	1	1	1	1	1	1	
200	1	1	1	1	1	1	
100	1	1	1	0	1	1	OP-M14

Absence of band : 0 band appearance: 1

7 - primer OP-T20

the results in Table (11) that the primer OP-T20 recorded a large number of total bands (38 bands) with a relatively high efficiency of 12.5%, which resulted in 8 link sites and five of them are identical. As it appeared in all cultivars at molecular weight (650, 550, 400, 375 and 300 bp), and two of them differed at molecular weight (500 and 75 bp), constituting a percentage of 37.5%,Which gave a discriminating ability of 11.53%, the primer did not find any sequences with the genome at the molecular weight of 225bp to the Buraq cultivar. Thus, it can be considered as a genetic imprint of this cultivar at this molecular weight in the event that work is completed on designing special primers for the duplicating region,

Table (11) The individual polymorphic and nonpolymorphic bands with their molecular weights for the primer OP-T20

molecular weights	**1**	**2**	**3**	**4**	**5**	**6**	
650	**1**	**1**	**1**	**1**	**1**	**1**	
550	**1**	**1**	**1**	**1**	**1**	**1**	**OP-T20**
500	**0**	**1**	**1**	**0**	**1**	**1**	
400	**1**	**1**	**1**	**1**	**1**	**1**	

375	**1**	**1**	**1**	**1**	**1**	**1**	
300	**1**	**1**	**1**	**1**	**1**	**1**	
225	**0**	**1**	**0**	**0**	**0**	**0**	
75	**1**	**1**	**0**	**0**	**0**	**1**	

Absence of band : 0 band appearance: 1

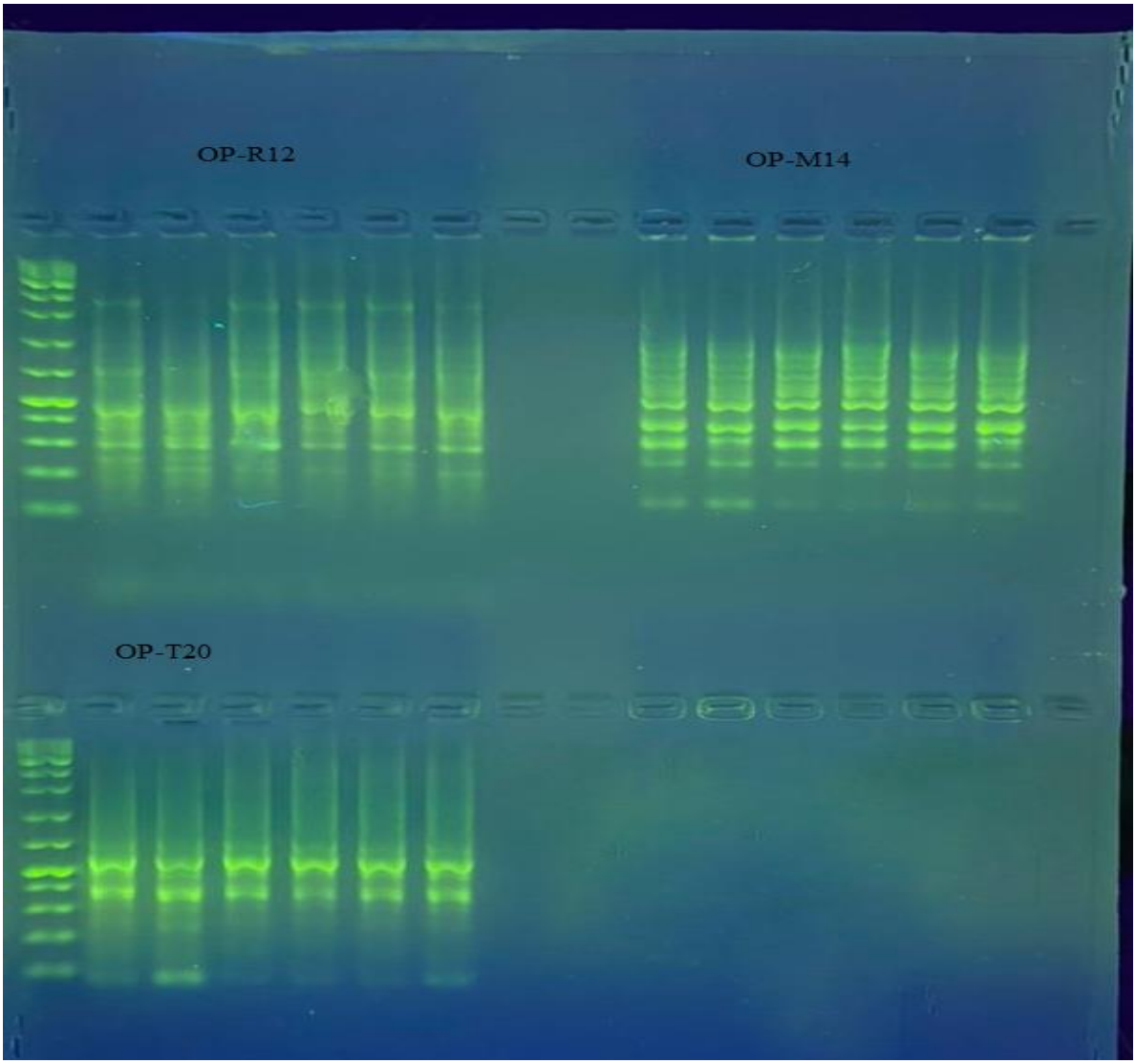

Figure (2) primer results (OP-T20,O P-R12, OP-M14) on 1% agarose gel

8 - primer OP-M20

This primer OP-M20 achieved a total number of 23 bands by linking to four alleles sites, and the efficiency of the primer was relatively low, which amounted to 6.25%, and the number of dissimilar bands amounted to only one and the polymorphism of the primer amounted to 25%, which reflected that number on the discriminatory ability of the primer , which amounted to 3.84%, at the same time that this primer was able to identify its

complementary sequences in the DNA of the cultivars at the molecular weights (1000, 450 and 375 bp).This indicates the identification of its sequences in these cultivars, while one bands appeared absent at the molecular weight of 1300 pb according to the results of Table (12)In addition, the appearance of the only bands in this cultivar without the other cultivars may be due to the discrepancy of the parents from which this cultivar is descended and with a difference in genetic structure compared to other cultivar.

Table (12) The individual polymorphic and nonpolymorphic bands with their molecular weights for the primer OP-M20

molecular weights	1	2	3	4	5	6	
1300	1	0	1	1	1	1	OP-M20
1000	1	1	1	1	1	1	
450	1	1	1	1	1	1	
375	1	1	1	1	1	1	

Absence of band : 0 band appearance: 1

9 - primer OP-V19

the results in Table (13) for the primer OP-V19 that 17 duplicative segments appear on the agarose gel, with an efficiency of 4.68%, and a binding at 3 alleles sites, two of which are the same in all cultivars as main bands at the molecular sites (350 and 275bp),Waving so that it found his own sequences in these cultivars .This primer was able to identify the complementary sequences in the genome DNA of the species ,It achieved a clear position difference at the molecular weight (200bp) in the presence of one absent band, and a percentage of different bands (1%), with an efficiency of 4.68% and a discriminatory ability (3.84%).

Table (13) The individual polymorphic and nonpolymorphic bands with their molecular weights for the primer OP-V19

molecular weights	1	2	3	4	5	6	
350	1	1	1	1	1	1	OP-V19
275	1	1	1	1	1	1	
200	1	1	1	1	0	1	

Absence of band : 0 band appearance: 1

10-primer OP-M16

This primer OP-M16 achieved a total number of 21 bands by linking to four nocturnal sites, and the primer efficiency was relatively low (6.25%) and the number of the polymorphic bands was only two, and the polymorphism of the primer was 50%, This number was reflected in the discriminatory ability of the initiator

(7.69%), at a time when the primer was able to identify these complementary sequences in the DNA of the cultivars at molecular weights 550 and 1400 bp, according to what is proven in Table (14).

Table (14) The individual polymorphic and nonpolymorphic bands with their molecular weights for the primer OP-M16

molecular weights	1	2	3	4	5	6	
1400	0	1	1	1	1	1	
1200	1	1	1	1	1	1	
800	1	0	1	0	1	1	
550	1	1	1	1	1	1	OP-M16

Absence of band : 0 band appearance: 1

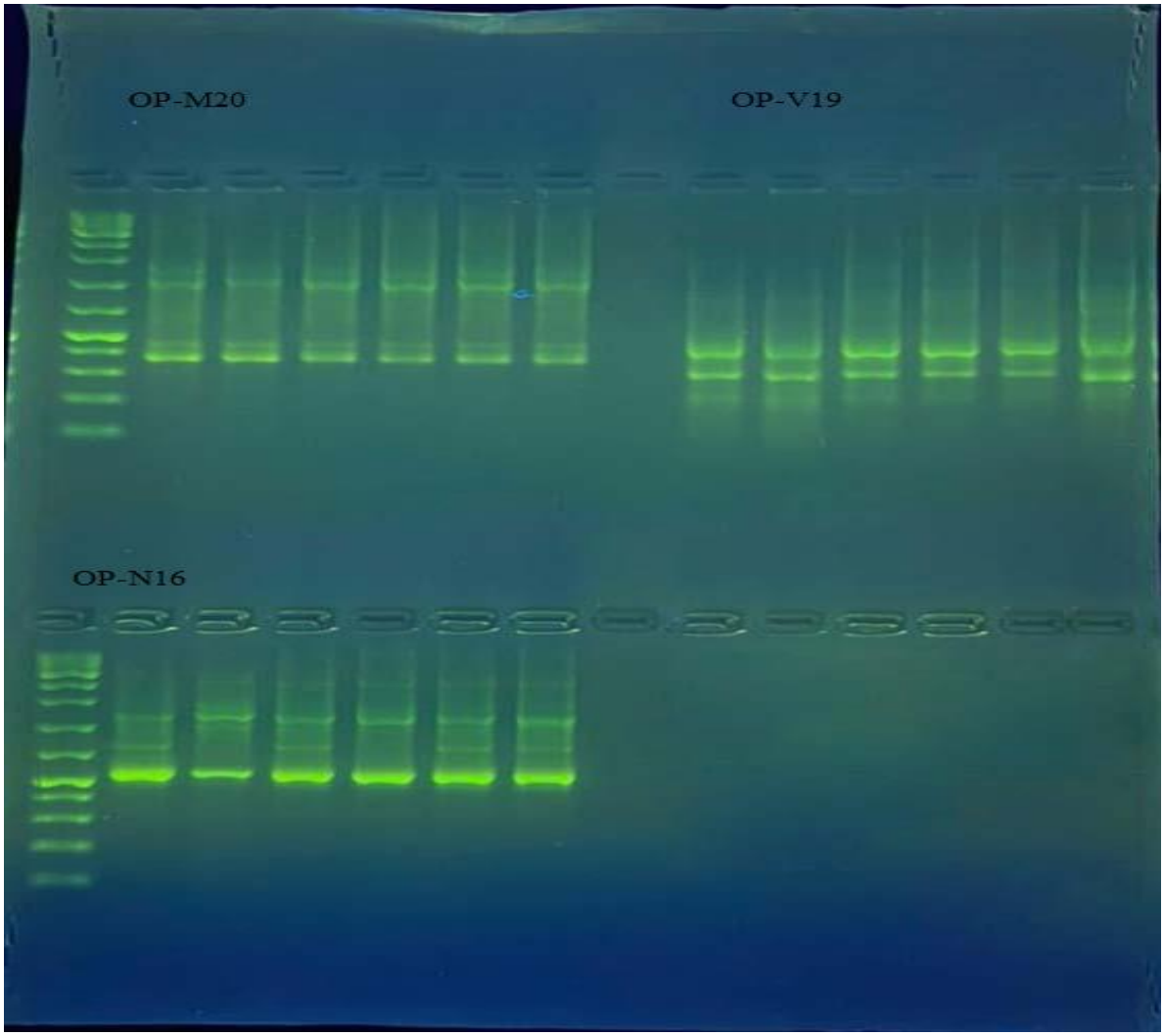

Figure (1) primer results(OP-N16, P-V19, OP-M20) on 1% agarose gel

It is clear from the above that the results obtained with RAPD-PCR technique have proven successful in finding a discrepancy between the cultivars under study even if the distinction is not apparent between them, and that the ten initiators have all succeeded in double DNA and for all the cultivars under study, This is because all of them found complementary binding regions for the sequence of nitrogenous bases in the DNA of the genome of the studied cultivars. Some of them produced a large number of bands, while others produced a smaller number of bands, and the emergence of a high percentage of genetic variance between cultivars is evidence of the effectiveness of the RAPD technique in finding genetic variance between genotypes even if there is a slight difference between them. These results are consistent with the results of Awaad et al. (2010), Zakaria (2011), Benabdelhafid et al (2015) and Anis and Al Dulaimi (2020), which demonstrated the success of RAPD technique in finding genetic variations between a group of forage crop cultivars.

4-3-2: Genetic distance Values Among cultivars Using RAPD Technology

The genetic distance coefficient was calculated between the cultivars under study based on the results that showed the presence of 26 polymorphic bands using NTSYS-pc program and based on the Hamming Similarity Index shown in Table (15) and the highest percentage of genetic similarity (lowest genetic distance) was 0.027 between the Alhadr and Amal cultivars of sequence (5 and 3), as they formed a very small group compared to the rest of the cultivars and that all the closely related cultivars are local cultivars, naturalized and approved by the Ministry of Agriculture, and this is due to their participation in a small number of bands due to the presence of differences in the nucleotide sequences in the genome of these two cultivars. Here, the role of using these different primers that target several regions in the gene becomes clear, thus showing the differences between these cultivars used according to the sequence of the primer used (Yassin, 2011). While the highest genetic distance of 0.188 was found between the two cultivars (shueae and Buraq) with sequences (4 and 2), followed by the genetic distance between the cultivars (Buraq and IPA 99), (Buraq and Alhadr) and (Buraq and Amal) with sequences (2, 6 and) 2, 5), (2 and 3) as it reached 0.185, 0.174 and 0.160 respectively, Perhaps the reason for the genetic diversity between them is due to their sharing of a small number of bands, in addition to the difference in the sequence of nitrogenous bases in the genome of these cultivars. This enables us to cross-breed between them to obtain a hybrid and then conduct selection to reach a promising cultivar with high productivity and at the same time exclude plants with low efficiency, and these results are consistent with the results of Yassin (2011), Cifci and Yagdi (2012), Al-Jubouri (2013) and Jerodia (2015), Al-Tamimi (2019), Al-Majma'i, and Anis (2020).

Table (15) the genetic distance of the cultivars included in the study

	1	2	3	4	5	6
1	0					
2	0.129	0				
3	0.109	0.160	0			
4	0.134	0.188	0.055	0		
5	0.102	0.174	0.027	0.066	0	

6	0.132	0.185	0.0545	0.057	0.065	0

4-3-3: Drawing the genetic tree of cultivars based on RAPD

A genetic dendrogram is a diagram showing the evolutionary relationships of a group of organisms that originate from a common ancestor, and is in the trunk of the tree, The organisms that originate from it and are found at the end of the tree branches, and individuals may be different with each other in appearance, but they are genetically close to each other, and therefore these differences may be due to environmental influences only to be taken into account in determining the degree of genetic dendrogram between individuals. The selection of parents and prediction of the best hybrids and limiting them to a few varieties that contain the largest possible number of genetic classifications are useful in future plant breeding and improvement programs.

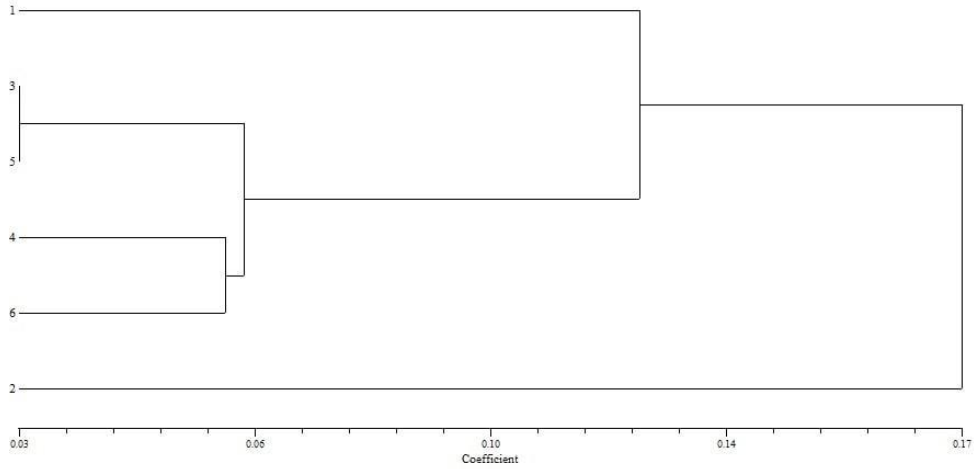

Figure (4) genetic relationships analysis of the studied cultivars

Where the cluster analysis of the cultivars included in this study using RAPD indicators showed that they were separated into two main groups, the first represented by the Buraq cultivar and the second group for the rest of the cultivars with a distance of 0.17 and at the same time the second main group was also divided into two secondary groups with a distance of 0.13,Where the cultivar IPA 99 was isolated in one independent group, while the second secondary group was in turn divided into two groups with a distance of 0.06,The first included the two cultivars Amal and Alhadr, of sequence (3) and (5), and the second of the two cultivars Shuaa and IPA 99, of sequence (4) and (6).It is clear from the above that the Buraq cultivar with sequence (2) was the farthest genetically from the rest of the cultivars, followed by the Samir cultivar with sequence (1), and thus, it is possible to invest in the Buraq cultivar (with excelled phenotypic effects) and cross it with the rest of the cultivars included in the study and other cultivars that were not included in the study. This study is intended to be used in the future to obtain a hybrid strength for the yield trait or any other trait that characterizes these structures, and to stay away from cross-breeding of genetically close cultivars with a high percentage of similarity because it leads to the accumulation of unwanted genes,In addition, some of the genetic material of

these cultivars may be similar according to the number of DNA segments of the genome of these varieties complementing the sequences of the primers used in this study or show the extent of their association with each other, and these results are consistent with the results of Zakaria (2011) and AL-Hamadny et al. (2014) and Al-Karkhi (2018) and Anis Al-Dulaimi (2020).We conclude from the above that the use of the RAPD technique, which is distinguished by its low cost, ease and effectiveness in distributing cultivars into groups according to genetic origin, so that we can selection parents with a broad genetic base, especially the two varieties Buraq and Shahah, and benefit from them in the genetic improvement and breeding programs.

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