Tikrit Journal for Agricultural Sciences (2023) 23 (4): 176-191
DOI: https://doi.org/10.25130/tjas.23.4.14

IRAQI Academic Scientific Journals
العراقية المجلات الأكاديمية العلمية

ISSN:1813-1646 (Print); 2664-0597 (Online)
Tikrit Journal for Agricultural Sciences
Journal Homepage: http://www.tjas.org
E-mail: tjas@tu.edu.iq

TJAS
Tikrit Journal for Agricultural Sciences

Genetic Distance for Genotype Barley (*Hordeum vulgare* L.) using RAPD-PCR Technology

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KEY WORDS:
Barley; Genotypes; Technology RAPD-PCR

Received: 29/05/2022
Accepted: 17/09/2023
Available online: 31/12/2023

ABSTRACT

The study targets to know the genetic Distance of fifteen genotypes of six-row barley (Ibaa99 , Alhadar , Alkheer , Alwarkaa , Buraq , Amal , Shuaa , Rehan , Samir , Arevate , Acsad1811 , Acsad1816 , Acsad1818 , Acsad1827 , Acsad1840) Based on the RAPD technique using fifteen primers, The primers used produced 111 band, including 94 band for variant alleles, with a rate of 84.68%, The molecular weights of the prefixes ranged between 200-2000 bp,. The primer OPB-14 gave the highest number of variant sites, reaching 10 bands, and a high discriminatory ability of the primer, which amounted to 10.64, the highest percentage of variant alleles in the primers (OP B-14, OP C-08, OP D-03, OP D-18, and OP F-05) was (100%), and the primer OP C-16 gave the highest efficiency of 11.209. The genetic divergence values showed that the largest genetic divergence was between the Alkheer cultivar and the Acsad1840 genotype (49.00), while the least genetic divergence was between the two genotypes Acsad1818 and Acsad1827, with a value of (9.00), In addition, the results of the kinship tree analysis of the genotypes were divided into two main groups, where Ibaa99 were in the first main group, while the other genotypes were in the second main group. From the results obtained, it is possible to rely on genetically divergent structures in breeding programs by introducing them into crosses with other varieties to obtain good productive characteristics.

البعد الوراثي لتراكيب وراثية من الشعير (*Hordeum vulgare* L.) باستخدام تقنية الـRAPD-PCR

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الخلاصة

تهدف هذه الدراسة الى معرفة البعد الوراثي لخمسة عشر تركيبا وراثيا من الشعير سداسية الصف (اباء99، الحضر ، الخير ، الوركاء ، براق ،امل ، شعاع ، ريحان ،سمير، اريفات ، ACSAD 1811، ACSAD 1816، ACSAD 1818، ACSAD 1827، ACSAD 1840)باستخدام خمسة عشر بادئا، وانتجت البادئات المستخدمة 111 موقعا للارتباط من ضمنها 94 موقع للاليلات المتباينة وبنسبة 84.68%، وتراوحت الاوزان الجزيئية للبادئات بين 200-2000bp، حيث اعطى البادئ OPB-14 اعلى عدد لمواقع الارتباط المتباينة وبلغ 10حزم وبقدرة تميزية عالية بلغت 10.64، وكانت اعلى نسبة الاليلات المتباينة في البادئات (OP B-14 و OP C-08 و OP D-03وOP D-18 و OP F-05) بلغت (100%) و اعطى البادئ OP C-16 اعلى كفاءة بلغت 11.209. كان اكبر تباعد وراثي بين الصنف الخير والتركيب الوراثيAcsad1840 (49.00)في حين كان اقل بعد وراثي بين التركيبين الوراثيين Acsad1818 و Acsad1827 بلغ (9.00)، اضافة الى ذلك فان نتائج تحليل شجرة القرابة الوراثية للتراكيب انقسمت الى مجموعتين رئيسيتين حيث انفرد الصنف اباء 99 في المجموعة الرئيسية الاولى بينما كانت التراكيب الاخرى في المجموعة الرئيسية الثانية، وبذلك فانه يمكن الاعتماد على التراكيب المتباعدة وراثيا في برامج التربية من خلال ادخالها في تهجينات مع اصناف اخرى للحصول على صفات ذو انتاجية عالية.

الكلمات المفتاحية: البعد الوراثي، شعير، RAPD-PCR.

INTRODUCTION

Barley (*Hordeum vulgare* L.) which belongs to the Poaceae family, is considered one of the important agricultural crops in the world and ranks fourth in terms of economic importance after wheat, maize and rice. In Iraq, it is considered the second crop after wheat in terms of the cultivated area (3092 thousand dunums).) and productivity (267 thousand tons) according to the 2021 statistics (Central Bureau of Statistics 2021). Its importance comes from its use in many fields, as it can be ground to be used in making bread after mixing it with wheat flour. Its grains contain 10-12.2% protein, 75.6-77.2% starch, 1.5-2.4% fat, and 4.6-5.2% ash (FAOstat,2010).

The barley crop when cultivated faces many problems, including lack of production, which is usually due to several factors, including varieties, seed rate, the nature of the soil, fertilizers, the problem of lying down and other determinants. Varieties with good productivity, and there are other varieties with little productivity, but they are distinguished by some characteristics that distinguish them from good varieties, such as resistance to insects and diseases, drought tolerance, resistance to lodging, and others. And that the quantities of seeds also determine the productivity of the variety, as when using small quantities of seeds, the growth of bushes leads to this crop, which causes economic loss and poor quality of the product, while the use of higher quantities than required leads to the competition of the plant for nutrients and light. And that the quantities of seeds also determine the productivity of the variety, as when using small seed rate, the growth of the associated weeds this crop leads to economic loss and reduced quality of the product, while the use of seed rate higher than

required leads to the competition of the plant for nutrients and light. Molecular markers are important in plant breeding programs because of the possibility of identifying a genetic location required for a specific genotype and not being affected by the phenotypic form of the plant, unlike the morphological and productivity indicators, in addition to obtaining a large number of indicators in a short time, and these indicators are the RFLP, AFLP, SSR, ISSR, and RAPD Which has proven its effectiveness in the last three decades, which led to its widespread use alone or in combination with other physiological and productive indicators. The technique of RAPD (Random Amplified Polymorphic DNA) is used in the identification of images, multiple phenotypes, genetic differences, the study of phylogenetic relationships, the evolution of species, and the determination of pedigree in a wide range of plant species, as well as its increasing importance as it is one of the indicators that depend on PCR in its work. The basis for the work of this technique depends on the presence of random primers associated with sites that are compatible with their bases and do not need to specify the nucleotide sequence (Zakaria,2011). Among the previous studies that dealt with this topic is the study of Al-Ati (2013)when studying the phenotypic and molecular indicators of fourteen genotypes of bread wheat using the RAPD technique, 19 out of 23 primers gave results for doubling, and the molecular weights ranged between 240-2000 bp, and the two primers (OPB-19 and OPI-06) gave 15 bands, while the primer (OPQ-20) gave 58 bands.

The results presented by Anees (2015) to study the effect of the genetic distance relationship in the cluster analysis of eight promising wheat strains were also shown, as the results of the cluster analysis showed that it was divided into five main groups, In the first main group there was genotype (8), in the second group there were genotypes (7 and 9), while the third group included genotype(6), and in the fourth group there was genotype(1), and finally the fifth group, which was divided into two secondary groups, the first was genotype(4) and the second was genotypes (2, 3, and 5). In a study by Olgun et al (2015) using 12 primers, the total number of bands was 95 and the number of different bands was 89 with a rate of 93.6%. Molecular weights ranged from 250 bp to 3200 bp, the primer OPA-4 gave the highest number of bands 11 bands while OPBB-3 gave the least number of bands 4 bands, The results of the cluster analysis showed that the barley varieties (12 varieties) were divided into three main groups. The first group included (Martı, Erginel-90 and Kral), and the second group, which contains five genotypes, was also divided into two subgroups, in addition to that the greatest similarity was observed between Çıldır-02 and Bolayir (0.164), and the largest difference between the genotypes Beyşehir-98 and Kral-97 and it reached (0.812). The results of Al-Karkhi et al (2018) showed when they studied seventeen genotypes of bread wheat using 23 primers, which gave 1327 total bands resulting from 142 link sites, including 127 different alleles and 15 major alleles, and the sizes of the duplicated bands for all primers ranged from (140-1500bp) The high polymorphism coefficient value, which ranged between 7756.0 and 9226.0, indicated an increase in the discriminatory ability of the primers used, and that the value of diversity gene ranged between 8028.0 and 9273.0, and the largest genetic distance was between the genotype JIHAN 99 and the genotype ZENGIRCI, and it amounted to 791.0 , while the lowest genetic distance was between the two genotypes TURKMEN and Gerek, amounting to 318.0.

The genotypes in the genetic kinship tree were distributed into three main groups. Based on the foregoing, the study aims to know the genetic diversity among 15 genotypes of barley, based on the RAPD technique, to be used in future breeding programs.

MATERIAL AND METHODS

A field experiment was conducted to study the genetic distance in barley in the Tikrit Research Dept-Agricultural Research Dept - Ministry of Agriculture, to study the genetic distance of (15) genotypes of barley (six-row), the details of which are shown in Table (1) using the RAPD technique, where the land was prepared It is designated for cultivation after it was plowed, smoothed, leveled, and cut into sheets with distances of (2 * 1) m. The experimental unit contained 5 lines of 2 m in length, and the distance between one line and another was 0.2 m. The varieties and genotypes were planted on 20/11/2022, and all crop service operations were carried out according to Recommendations.

Table (1) Genotypes used in the experiment

NO.	Genotypes	Pedigree	Source
1	Ibaa99	OAP-4AP-7L,sel /ICARDA	Agriculture R. Center
2	Alhadar	Radiation local black x Arevat-IRAQ	Atomic Energy
3	Alkheer	Radiation local black x Arevat-IRAQ	Atomic Energy
4	Alwarkaa	-	Atomic Energy
5	Buraq	-	Atomic Energy
6	Amal	Irradiation of Nomar cultivar with	Atomic Energy
7	Shuaa	Radiation Arevat seed IRAQ	Atomic Energy
8	Rehan	Radiation Arevat seed IRAQ	Agriculture R. Center
9	Samir	Radiation local black x Arevat-IRAQ	Atomic Energy
10	Arevate	-	Atomic Energy
11	ACSAD 1811	-	ACSAD
12	ACSAD 1816	-	ACSAD
13	ACSAD 1818	-	ACSAD
14	ACSAD 1827	-	ACSAD
15	ACSAD 1840	-	ACSAD

A laboratory experiment was conducted in the laboratory of the Department of Life Sciences (Molecular Genetics Laboratory) - College of Science - University of Tikrit, where DNA was extracted from young barley leaves using the CTAP method for all varieties and genotypes used in the experiment to obtain high-purity DNA. DNA by electrophoresis followed by staining with ethidium bromide and visualized by UV light. DNA samples were diluted to a concentration of 100 ng μl^{-1} in order to be used in RAPD-PCR experiments. 15 primers were used, the details of which are mentioned in Table (2). Random amplification reactions for DNA fragments (RAPD-PCR) were carried out according to Williams et al (1990) using the AccuPower PCR premix Kit prepared from the Korean company Bioneer, and according to the attached instructions. Each tube contains the basic components of the polymerase chain reaction, which include: 1 unit Taq DNA polymerase, 250 µM mixture of dNTPs ,10mM Tris-Hcl (pH 9) ،30mM KCL and 1.5 MgCl2 mM, And 10 picomole of the primer and 25 ng of DNA were added to it, then the reaction volume was completed with sterile distilled water 20 Lµ for each tube, the tubes were transferred to the thermocycler and the multiplication reaction was performed after it was programmed according to the program. One cycle for two minutes at 94°C for the initial deformation of the DNA strand, followed by 40 replication

cycles. Each cycle includes one minute at 92°C for template deformation, one minute at 36°C for binding the primers to the template DNA, and one minute at 72°C for elongation, with a final cycle of 2 minutes. 7 minutes at 72°C for final elongation. The products of the amplification process were transferred to the agarose gel at a concentration of 1.2% with the size index consisting of lambda DNA, which was cut with the Hind III enzyme and the EcoRI, for 90 minutes at (5V cm^{-1}), examining the gel after staining it with ethidium bromide for 30-45 minutes under UV-light and images using the Gel Documentation System, and according to the number of beams produced with their molecular sizes for each primer.

Table (2) Primers used in RAPD Reactions

No.	Primer code	Nucleotide sequence 5 to 3
1	OP A-01	CAGGCCCTTC
2	OP A-06	GGTCCCTGAC
3	OP B-14	TCCGCTCTGG
4	OP B-20	GGACCCTTAC
5	OP C-08	TGGACCGGTG
6	OP C-16	CACACTCCAG
7	OP D-03	GTCGCCGTCA
8	OP D-18	GAGAGCCAAC
9	OP E-03	CCAGATGCAC
10	OP E-11	GAGTCTCAGG
11	OP F-05	CCGAATTCCC
12	OP F-20	GGTCTAGAGG
13	OP G-08	TCACGTCCAC
14	OP G-14	GGATGAGACC
15	OP H-08	GAAACACCCC

The results of the RAPD primers were taken from its table and based on the comparison of the presence or absence of DNA segments for different samples, as the presence of the DNA segment is symbolized by the number (1) and its absence by the number (0). The genetic distance coefficient and the similarity coefficient between the studied taxa were calculated using Nei's coefficient (Nei and Li, 1979), cluster analysis was performed, and the genetic distance scheme was drawn using the UPGMA method (Sneath and Sokal, 1973), Statistical analyzes were carried out by computer using NTSYS_pc (Rohlf, 1993). And the conclusion of the results of this program was based on the Nei equation to detect genetic similarity by forming a sequential table that includes all the results of the primers (for each indicator separately) with all the studied models.

$$\text{Genetic Distance}=1-\{ 2x(Nxy / N x + N y)\}$$

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.

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 DISSCUSION

Although the RAPD technique is one of the old and relatively simple molecular techniques in detecting the genetic distance, it is still widely used due to its ease of application and the cheapness of its costs compared to other high-precision techniques (Mir Ali and Nabulsi, 2004). The results showed that the primers used all succeeded in doubling the DNA in all genotypes, despite the variation in the appearance of the bands at times, as it gave many bands in some primers, and at another time the number of bands was small, and this difference in the appearance of the bands resulted from the association of the primer with the structures hereditary sequences, And it confirms the existence of a genetic difference between the studied genotypes, which enhances the effectiveness of this technique in detecting the genetic Distance even in the case of its presence in low degrees, and these results are consistent with (Zakaria, 2011).

Table (3) Genetic variations of the primers used in the study

No.	primers	total number of bands	Monomorphic	Polymorphic	%Polymorphism	the discriminatory ability%	primer efficiency %	link sites	Molecular weights
1	OP A-01	82	1	7	88	7.45	8.063	8	400-1500
2	OP A-06	47	1	5	83	5.32	4.621	6	500-1500
3	OP B-14	87	0	10	100	10.64	8.555	10	400-2000
4	-20OP B	70	3	4	57	4.26	6.883	7	400-1500
5	OP C-08	40	0	4	100	4.26	3.933	4	600-1500
6	OP C-16	114	1	9	90	9.57	11.209	10	300-1500
7	OP D-03	44	0	8	100	8.51	4.326	8	400-1500
8	OP D-18	86	0	9	100	9.57	8.456	9	400- 1500
9	OP E-03	60	2	4	67	4.26	5.900	6	300- 2000
10	OP E-11	76	2	7	78	7.45	7.473	9	400-1500
11	OP F-05	66	0	8	100	8.51	6.490	8	500-1500
12	OP F-20	104	3	5	63	5.32	10.226	8	400-1500
13	OP G-08	32	2	2	50	2.13	3.147	4	300-1500
14	OP G-14	56	1	8	89	8.51	5.506	9	200-1500
15	OP H-08	53	1	4	80	4.26	5.211	5	500-1500
		1017	17	94				111	200-2000
			1.33	6.26				7.4	

The results showed in Table (3) that there are differences in the number of alleles depending on the primer used, where all the primers gave a number of bands that reached 1017 bands, and the primers showed varying ability in detecting genetic variation between the studied genotypes, as these primers gave (111) the site of the allele It was divided into 17 major alleles and 94 secondary alleles, which are used to determine the genetic linkage between genotypes. It is noted that the primers OP B-14 and OP C-16 gave the highest number of link sites, amounting to 10 sites, and both, while the primers OP C-08 and OP G-08 gave the least number of sites, amounting to 4 sites, and both, and the

primers OP B-14 gave the highest number of divergent alleles. 10 allele, while the primer OP G-08 gave the lowest number of variant alleles, and the proportion of variant alleles excelled in the primers OP B-14, OP C-08, OP D-03, OP D-18, and OP F-05, reaching 100%, while the primer OP F-05 gave The primer starter OP G-08 has the lowest rate of 0.57%.

Primer OP A-01

This primer was used to replicate the DNA of genotypes and resulted in 82 bands of primer binding in 8 sites, 1 of which is a major site and the remaining 7 divergent sites amounted to 88%. The primer showed variation in the DNA in position and molecular weight, which ranged from 400-1500 bp, Figure (1) shows that the band with a molecular weight of 1200 bp appeared in all genotypes as an indication of the presence of special sequences in these genotypes. The discriminatory ability of this primer was 7.45 and the efficiency of the primer was 8.063. This variation of the genotypes in the sites where the multiplying or resulting pieces are distributed may be due to the descent of these genotypes from societies that were subjected to severe electoral pressure or the difference in the genetic base from which these genotypes descended, if neglected or neglected Spontaneous genetic mutations were excluded due to their low recurrence rates (Zakaria, 2011).

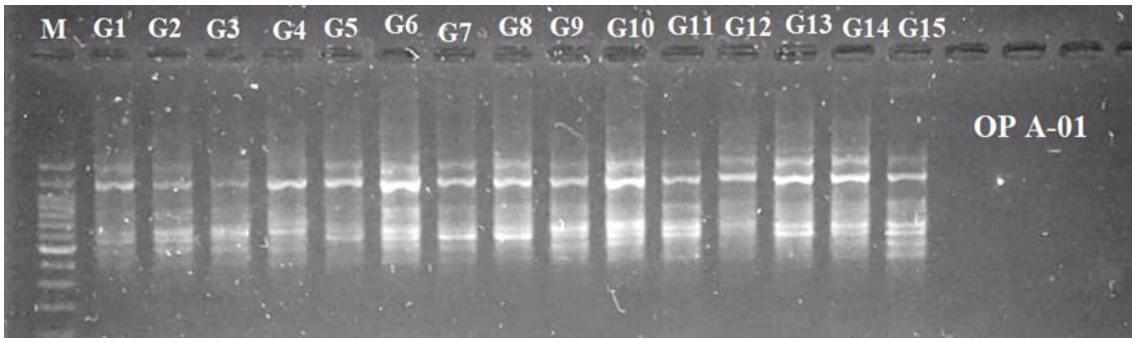


Figure (1) Results of primer electrophorese OP A-01 with DNA extracted from barley leaves

Primer OP A-06

It is clear from Figure (2) that this primer gave 47 total bands resulting from 6 allelic sites, as it was divided into one main site and 5 divergent sites, with a value of 83%. The molecular weights of this primer ranged between 500-1500. The results of the above figure showed that the 1200bp band appeared in all genotypes, and this indicates the presence of special sequences for it in these genotypes, and that the discriminatory ability reached 5.32 and the efficiency of the primer was 4.621. Sequences are complementary to it in this primer and vice versa. The presence of sequences in a specific genome and their absence in another genome is a criterion for the genetic divergence between the two genomes (Zakaria, 2011).

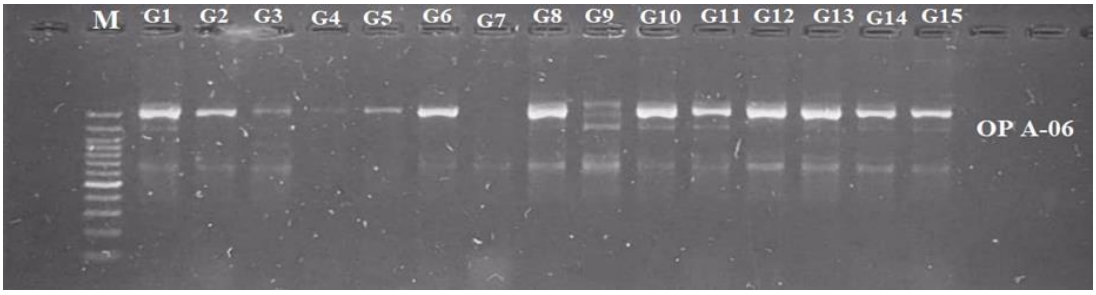


Figure (2) Results of primer electrophorese OP A-06 with DNA extracted from barley leaves

Primer OP B-14

The total number of bands for this primer reached 87, resulting in 10 alleles, all of which are divergent, so that the percentage of divergent bands is 100% (shown in Figure 3), as the primer was able to identify its complementary sequences in the DNA of the genome of the genotypes. It showed a clear difference in the molecular weight ranging between 400-2000 bp, and it is noted that this primer did not find the complementary sequence in the cultivar Samir, and thus it can be considered a genetic sign of this genotype in this primer, and the reason for the absence of bands may be due to the difference of its parents, and this primer has excelled in its ability discriminatory and efficiency of the was 10.64 and 8.555, respectively.

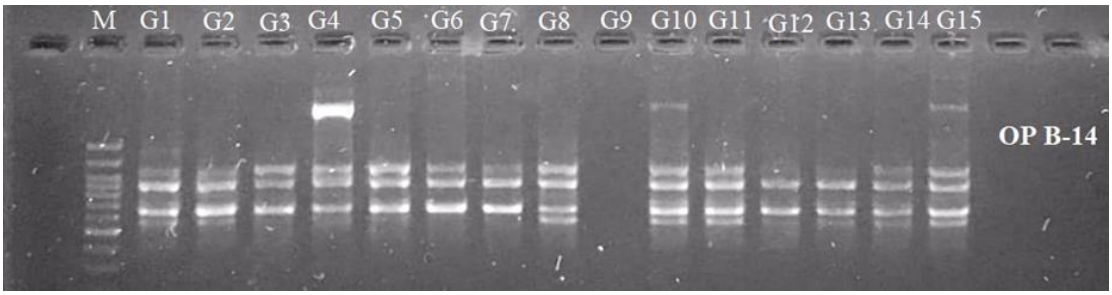


Figure (3) Results of primer electrophorese OP B-14 with DNA extracted from barley leaves

3Primer 20-OP B

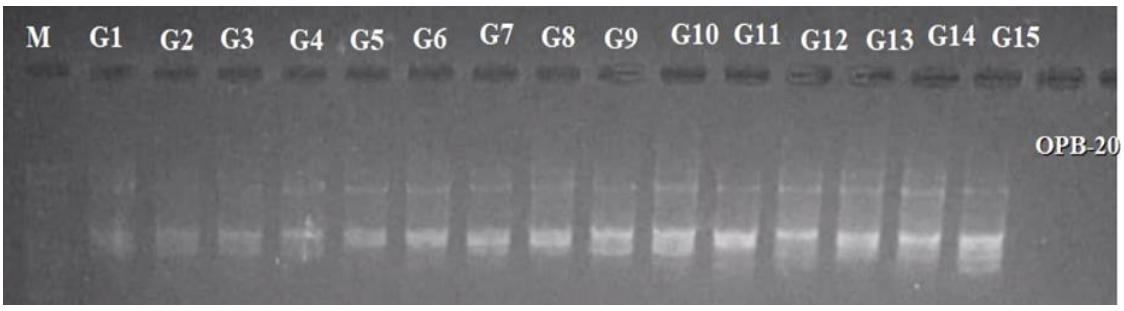


Figure (4) Results of primer electrophorese OPB-20 with DNA extracted from barley leaves

The results of Figure (4) showed the presence of 7 total bands that resulted in 7 link sites, of which 3 are major and four are 57% divergent, and the value of their molecular weights ranged between 400-1500bp, and the results showed that the bands 600, 700 and 1200pb were present in all genotypes.

This is an indication of the presence of its sequences in all genotypes, This primer gave a discriminative ability of 4.26 and an efficiency of 6.883.

Primer OP C-08

The results of Figure (5) indicate that this primer gave 40 bands, resulting in 4 binding sites that differed by 100%. The discriminant ability was 4.26 for this primer, its molecular weights ranged between 600-1500 bp, and this primer gave an efficiency of 3.933.

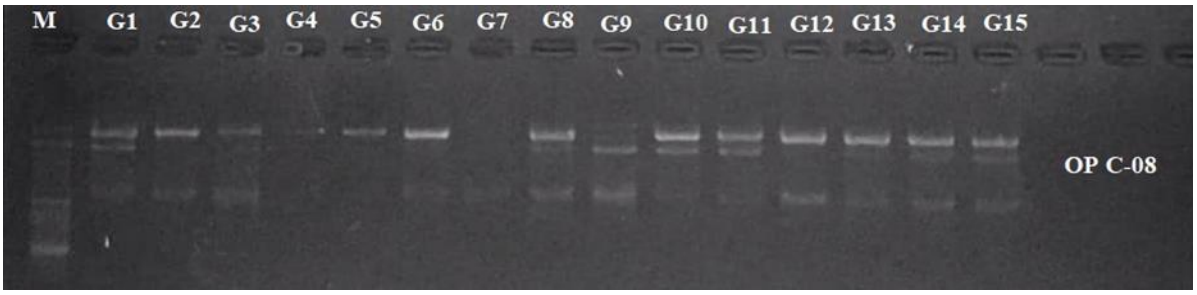


Figure (5) Results of primer electrophorese OP C-08 with DNA extracted from barley leaves

Primer OP C-16

The results of Figure (6) indicate that this primer has 10 binding sites, one of which is main and 9 divergent, to give 114 bands, with a rate of 90%, and a discrimination capacity of 9.57, The molecular weights of this primer ranged between 300-1500 bp, and the results showed that the 400 bp band was present in all genotypes, indicating the presence of its sequences in all genotypes. This primer gave the highest efficiency of 11.209.

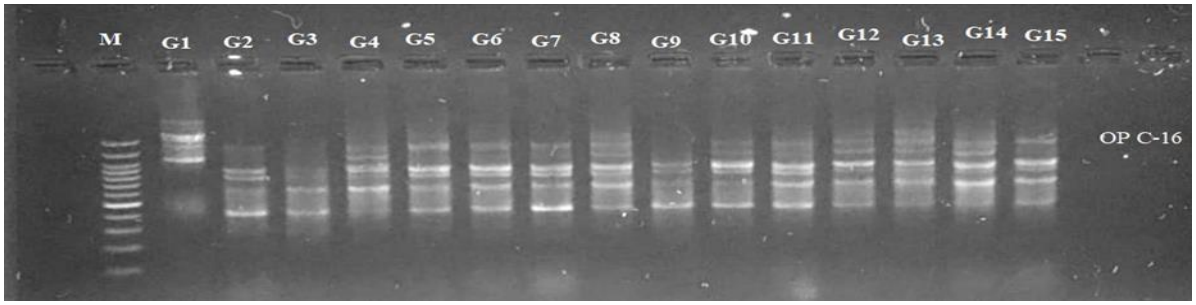


Figure (6) Results of primer electrophorese OP C-16 with DNA extracted from barley leaves

Primer OP D-03

This primer showed that the total number of bands resulting from its use is 44, resulting from 8 link sites, all of which are 100% different, with a discriminatory ability of 8.51, and an efficiency of 4.326 for the primer. The results of Figure (7) showed that the cultivar Amal did not give any band when using this primer, indicating the absence of the sequence complementing the primer in this genotype. Thus, it is considered a genetic imprint of the genotype in this primer, and the reason for the absence of the band may be due to the difference in the genotype parents, and the molecular weights of the primer ranged between 400-1500bp. The variation in the appearance of bands in some genotype and their absence in other genotype indicates the presence of complementary sequences of

this genotype in the primer and their absence in other genotype (Al –Majimai and Anees, 2020) and (Al-Karkhi et al, 2018).

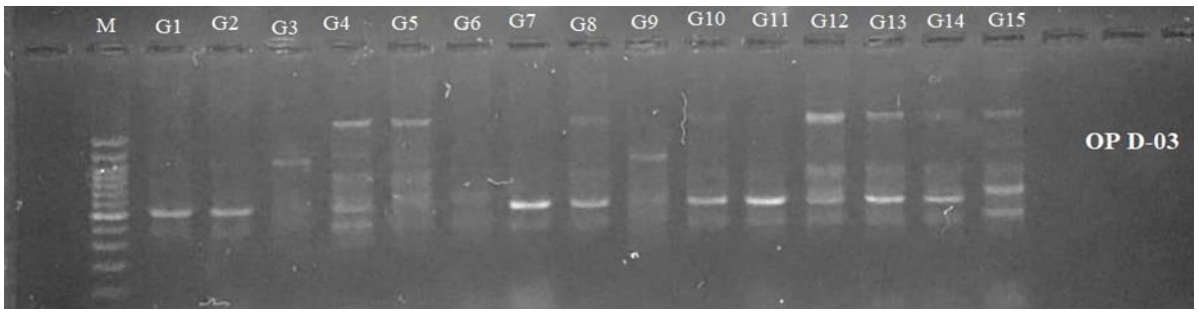


Figure (7) Results of primer electrophorese OP D-03 with DNA extracted from barley leaves

Primer OP D-18

It is clear from Figure (8) that the total number of bands resulting from the use of this primer is 86 bands, resulting from 9 link sites, all of which are 100% different bands. The appearance of the band in one genotype and its absence in other genotypes indicates the presence of complementary sequences for these genotypes in the primer and their absence in the other genotypes. The presence of sequences in a specific genome and their absence in another genome is a criterion for the genetic divergence between the two genomes, and these results agree with (Al-Ati, 2013). The discriminative ability of this primer was 4.26, and its molecular weights ranged between 400-1500 bp and its efficiency was 8.456.

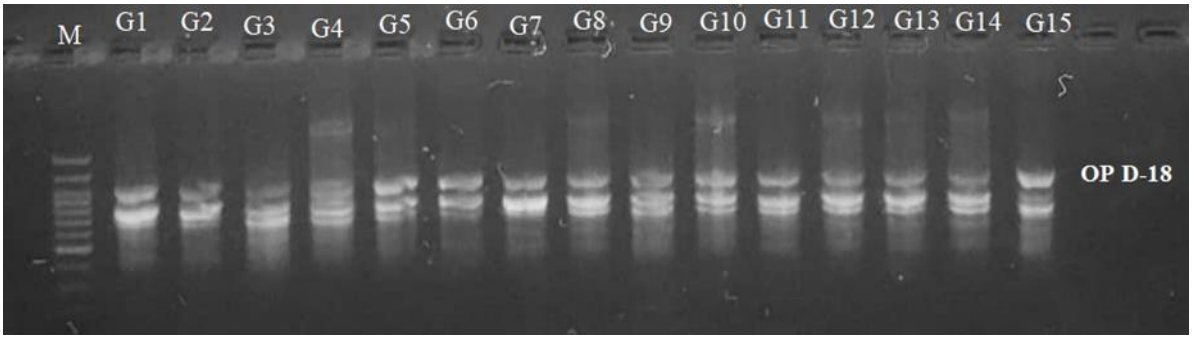


Figure (8) Results of primer electrophorese OP D-18 with DNA extracted from barley leaves

Primer OP E-03

The results of Figure (9) show that the molecular weights of this primer ranged between 300-2000 bp, and the total number of bands reached 60, resulting from 6 binding sites, 2 of which are major and 4 divergent, to be 67%, and that the 1200 and 2000 bp bands appeared in all genotypes. , an indication of the presence of the sequences of this primer in these genotypes, and the value of the discriminatory ability of the primer and its efficiency were 4.26, 5.900, respectively.

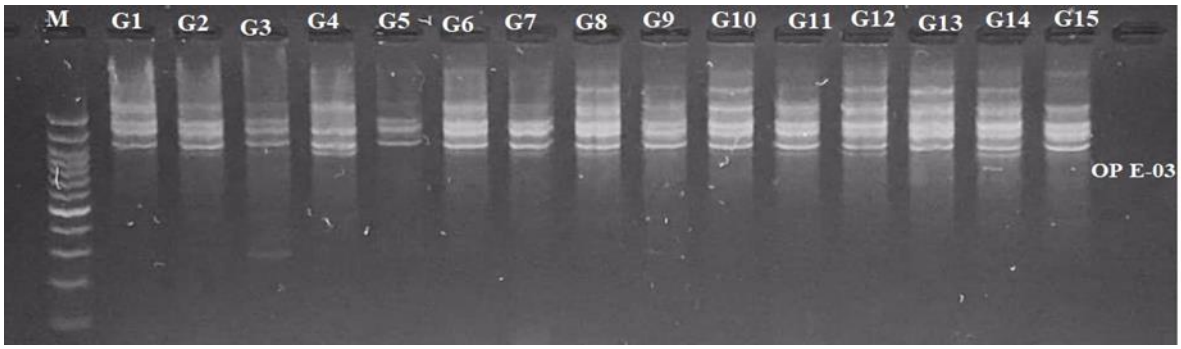


Figure (9) Results of primer electrophorese OP E-03 with DNA extracted from barley leaves

Primer OP E-11

When observing Figure (10), it is clear that the total number of bands for this primer amounted to 76 bands, which resulted in 9 link sites, 7 of which are different and 2 of which are major, and the Polymorphism was 78%. The molecular weights of this primer ranged between 400-1500 bp, and the bands 600 and 1200 bp appeared in all samples, indicating the presence of special sequences for this primer in all genotypes. The value of the discriminatory ability of the primer was 7.45 and the efficiency of the primer was 7.473.

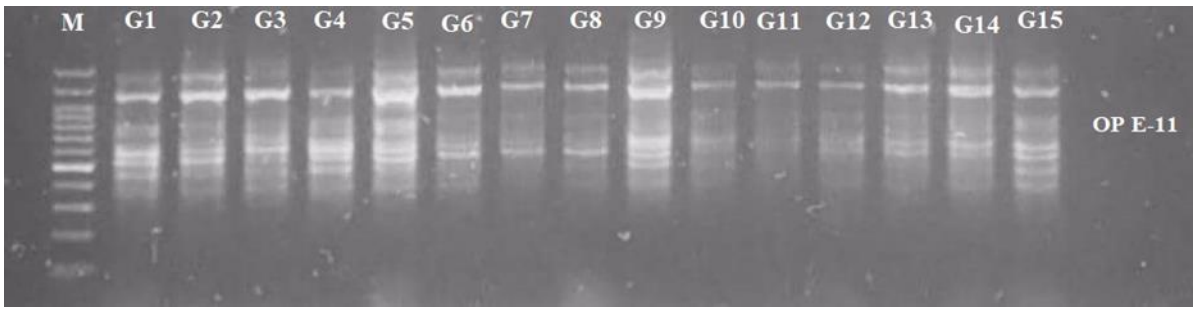


Figure (10) Results of primer electrophorese OP E-11 with DNA extracted from barley leaves

Primer OP F-05

The value of the discriminatory ability of this prime was 8.51 and its efficiency was 6.490, and the number of total bands reached 66 bands that resulted from 8 binding sites, all of which were 100% different, as shown in Figure (11), and the molecular weights ranged between 500-1500bp. The variation of the genotypes in the sites where the multiplying or resulting pieces are distributed may be due to the descent of these genotypes from societies that were subjected to severe electoral pressure or the difference in the genetic base from which these genotypes descended, if we neglect or exclude spontaneous genetic mutations due to the low frequency of their occurrence, and these agree results with (Al-Khazraji et al, 2022)

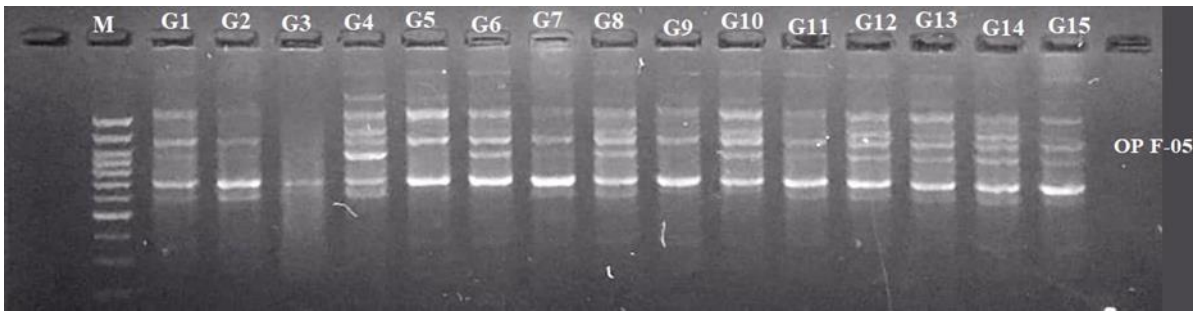


Figure (11) Results of primer electrophorese OP F-05 with DNA extracted from barley leaves

Primer OP F-20

The results of the molecular analysis of this primer in Figure (12) showed that the total bands of 104 bands resulted from 8 binding sites, 3 main and 5 different, Polymorphism 63%, and that the value of the discriminatory ability amounted to 5.32, and the molecular weights of this primer ranged from 400-1500bp, while the efficiency of the primer was 10.226 to come second in its ranking.

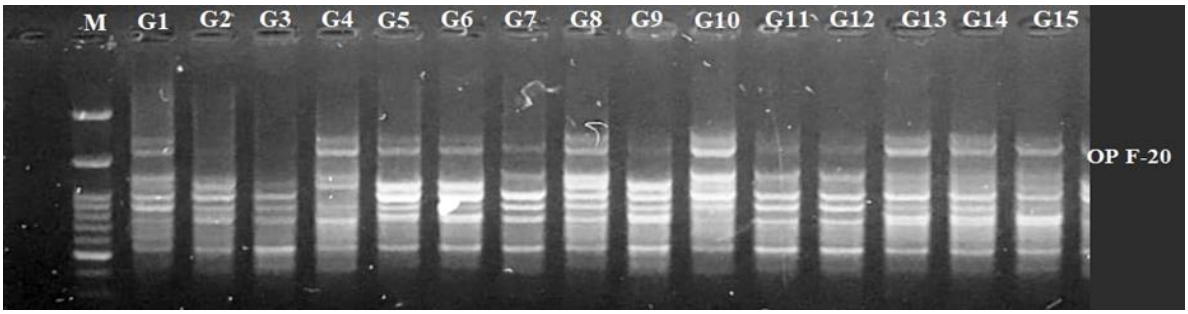


Figure (12) Results of primer electrophorese OP F-20 with DNA extracted from barley leaves

Primer OP G-08

It appears from Figure (13) that the binding sites for this primer amounted to 4 sites, including 2 main and 2 variant, to give the lowest values for the Polymorphism (50%), the discriminatory ability (2.13), the number of total bands (32), and the efficiency of the primer (3.147), and the molecular weights ranged Between 300-1500 pb, and also that the two bands 500 and 900 bp appeared in all structures, indicating the presence of special sequences for this primer in all genotypes.

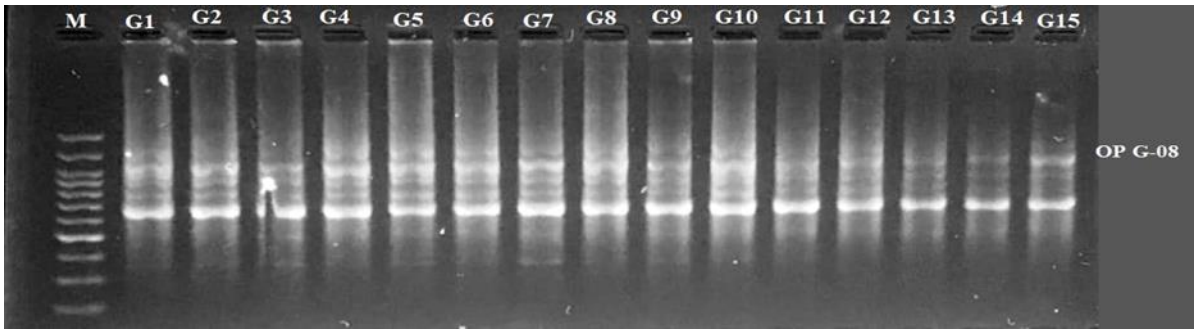


Figure (13) Results of primer electrophorese OP G-08 with DNA extracted from barley leaves

Primer OP G-14

The results of Figure (14) showed that the total number of bands for this primer amounted to 56 bands resulting from 9 link sites, one of which is main and 8 divergent, so that the Polymorphism is 89%. The discriminatory ability of this primer and its efficiency amounted to 8.51 and 5.506, respectively.

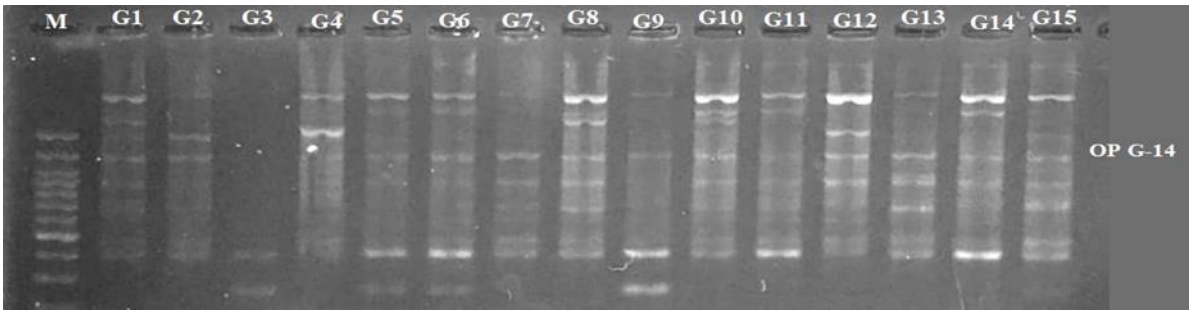


Figure (14) Results of primer electrophorese OP G-14 with DNA extracted from barley leaves

Primer OP H-08

The results of the molecular analysis show in Figure (15) that the total number of bands reached 53 bands to give 5 link sites, one of which is main and 4 variant, and the proportion of divergent alleles is 80%, and the discriminating ability reached 4.26, and the molecular weights of this primer ranged between 500-1500 bp, and the band is 500 bp. The samples showed an indication of the presence of the special sequences of this primer in all genotypes, while the efficiency of the primer was 5.211.

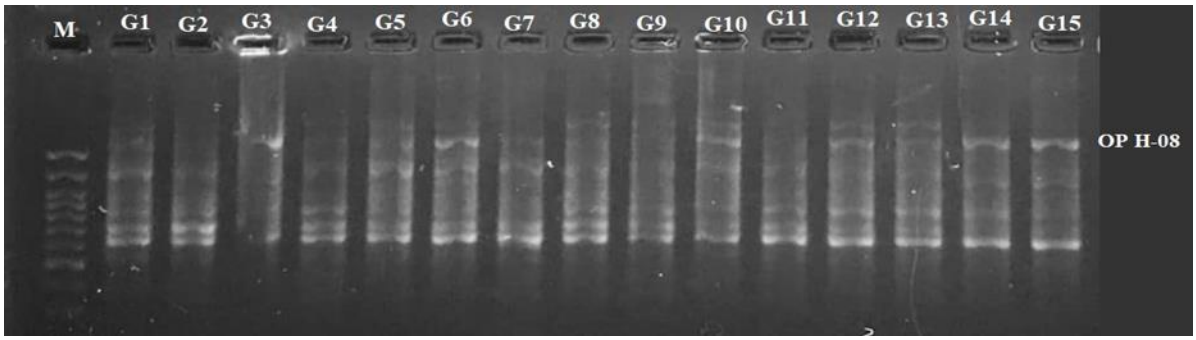


Figure (15) Results of primer electrophorese OP H-08 with DNA extracted from barley leaves

GENETIC DISTANCE

The genetic distance between genotypes was estimated based on the results of the RAPD analysis through the values of the common bands between the genotypes. Where the results of Table (4) show the variation in the values of the genetic distance, where the highest value of the genetic distance was between the Al-Khair cultivar and the genotypes Acsad1840, 1827 Acsad, 1818Acsad, and 1816Acsad (49.00, 47.00, 46.00, 45.00), respectively. The reason for the difference between the Al-Khair cultivar and the other genotypes may be due to their participation. In a few bands due to the difference in the nucleotide sequence in the genome of these genotypes, while the lowest value of the genotype was (9.00) between the two genotype Acsad1818 and Acsad1827, followed by the genotypes Acsad1816 and Acsad1818 (11.00). This difference is due to their participation in a small number of bands due to their difference in the sequence of nucleotides in the genome of these two genotypes, and here it is clear that the use of these different primers to target several regions of the genome, and thus shows the difference, if any, between the genotypes according to the sequence of the primer used. These results are consistent with what was found by(Al –Majimai et al 2020), (Al-Khazraji et al, 2022), (Anees 2015), (Fadel et al, 2022), (Olgun et al, 2015) and (Yaseen, 2011) .

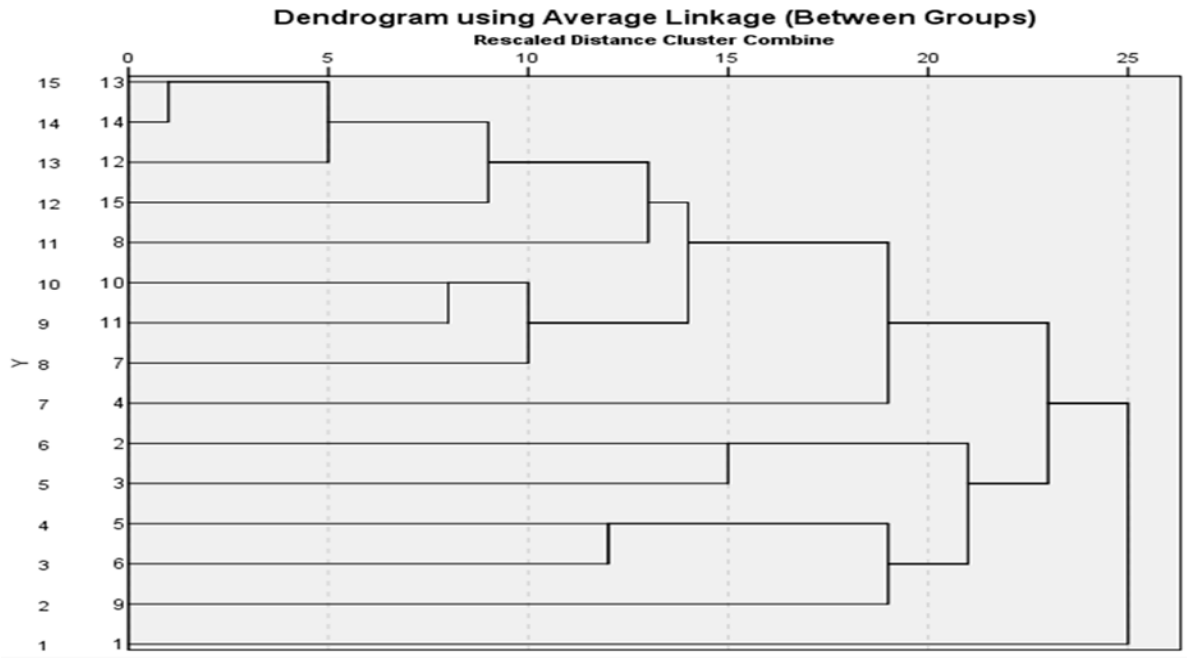
Table (4) Genetic distance values between barley genotypes

	Squared Euclidean Distance														
الاباء	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	.000														
2	28.00	.000													
3	43.00	25.00	.000												
4	44.00	28.00	43.000	.000											
5	42.00	34.00	35.000	24.00	.000										
6	36.00	26.00	27.000	38.00	22.000	.000									
7	31.00	21.00	32.000	31.00	29.000	21.000	.000								
8	31.00	29.00	42.000	33.00	35.000	29.000	28.00	.000							
9	42.00	36.00	31.000	38.00	32.000	26.000	31.00	41.00	.000						
10	33.00	31.00	42.000	33.00	37.000	29.000	22.00	22.00	35.00	.000					
11	32.00	24.00	35.000	28.00	38.000	24.000	17.00	21.00	28.00	17.00	.000				
12	38.00	30.00	45.000	26.00	28.000	32.000	23.00	25.00	38.00	21.00	26.00	.000			
13	39.00	33.00	46.000	25.00	27.000	27.000	24.00	20.00	33.00	20.00	23.00	11.00	.000		
14	34.00	28.00	47.000	26.00	34.000	30.000	27.00	19.00	38.00	21.00	24.00	16.00	9.000	.000	
15	38.00	36.00	49.000	30.00	34.000	34.000	27.00	25.00	40.00	21.00	26.00	22.00	19.00	14.000	.000

1. Ibaa99, 2. Alhadar, 3. Al-Khair, 4. Warka, 5. Buraq, 6. Amal, 7. Shuaa,8. Rayhan, 9. Samir, 10. Arifat, 11. ACSAD 1811, 12. ACSAD 1816,13. ACSAD 1818, 14. ACSAD 1827,15. ACSAD 1840.

Cluster analysis or Dendrogram is a chart that shows the evolutionary relationship of a group of organisms that arose from common descendants. Its importance is due to determining the genetic relationship using cluster analysis, as well as to the possibility of organizing genetic assets, choosing the parents included in the breeding programs, predicting the best hybrids, and knowing the least possible number of genotypes that contain the largest possible amount of genetic classifications in plant breeding and improvement programs, where the results showed Cluster analysis in Figure (16). The genotypes were distributed into two main groups at a distance of 0.25, where the cultivar Ibaa99 (1) was isolated in the first group, while the rest of the genotypes were in the second main group, which in turn was divided into two secondary groups, at a distance of 0.23. The first secondary group included two groups, and in turn was divided into two parts. At a distance of 0.21, the first group branched at a distance of 0.19 into two groups, the first branch of which is the variety Samir(9) , and the second branch also branched into two branches at a distance of 0.12, the two types, Buraq and Amal (6 and 5). The second group, at 0.15, was divided into two categories, Alhadar and Al-Khair (3 and 2). The second secondary group was divided at a distance of 0.19 into two branches, the first branch was the Warka variety (4) alone, while the second branch branched into two other parts at a distance of 0.14. Two secondary branches, the first branch was for the cultivar Rayhan (7) and the second secondary branch was divided into two other parts at a distance of 0.8, one of them for the Arifat variety (10) and the other for the composition Acsad 1811 (11), and the other branch in turn branched into two secondary branches at a distance of 0.9 The first secondary branch for the composition Acsad1840 (15), The second secondary branch was divided into two parts at 0.5, the first is the combination Acsad1816 (12), and the other branch also split into two parts at 0.1, the

genotypes Acsad1818 and Acsad1827 (13,14), and these results indicate that the genotype Ibaa99 was the farthest genetically, which enhances the opportunity to use it in future breeding programs. Also, a part of the genetic material of these genotypes may be similar according to the DNA segments of the genome of these genetic genotypes that complement 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 (Anees, 2015), (Olgun et al, 2015) and (Al-Khazraji et al, 2022).



1. Ibaa99, 2. Alhadar, 3. Al-Khair, 4. Warka, 5. Buraq, 6. Amal, 7. Shuaa,8. Rayhan, 9. Samir, 10. Arifat, 11. ACSAD 1811, 12. ACSAD 1816,13. ACSAD 1818, 14. ACSAD 1827,15. ACSAD 1840.

CONCLUSION

The greatest genetic distance was recorded between the Al-Khair cultivar and the ACSAD1840 genotype, amounting to, while the lowest genetic distance between the two genotypes ACSAD1818 and ACSAD1827.

ACKNOWLEDGMENTS

I extend my thanks and appreciation to the Department of Field Crops and the College of Agriculture at the Tikrit University.

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