Genetic Diversity, Relationship Determination, and Genetic Fingerprinting of Some Local Wheat Cultivars Cultivated in Salah al-Din Governorate Using RAPD-PCR Markers

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Abstract

The study aimed to assess the level of genetic diversity, determine relationships, and establish genetic fingerprints for eleven local bread wheat cultivars cultivated in Salah al-Din Governorate using 21 RAPD-PCR primers. Molecular bands were recorded as a binary matrix, where the presence of a band was coded as 1 and its absence as 0. Genetic distances were calculated according, and a dendrogram was constructed using the UPGMA method. The primers generated 169 loci, of which 124 were polymorphic and 45 were monomorphic, with a total polymorphism percentage of 73.37%. The total number of bands was 1300, comprising 805 polymorphic bands, 495 monomorphic bands, in addition to 13 unique bands and 32 absent bands. Unique bands helped distinguish six cultivars, with the cultivar 'Dhahab' recording five unique bands, followed by 'Mawaddah' with four bands. Genetic distance values ranged between 0.019 and 0.424; the highest similarity was observed between 'Fayyad' and 'Ibaa 99', while the greatest divergence was recorded between 'Mawaddah' and 'Sulaymaniyah'. Cluster analysis divided the cultivars into two main groups; the first group exclusively contained 'Mawaddah', while the second included the remaining cultivars. The results confirm the efficiency of RAPD-PCR markers in detecting molecular variation, distinguishing local cultivars, and providing useful information for genetic resource conservation and wheat breeding programs.

Keywords: Bread wheat, RAPD-PCR, Genetic diversity, Genetic fingerprinting, Genetic distance, UPGMA.

Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most prominent strategic cereal crops globally, serving as a primary source of food, energy, protein, and nutrients for large segments of the population. Bread wheat is characterized by a complex genetic composition, being an allohexaploid species encompassing three sub-genomes (A, B, and D), along with a large genome size and a high proportion of repetitive sequences. Genomic studies have revealed structural variations, allelic variations, and differences in gene content among its genetic constitutions, making the characterization of this diversity essential for understanding genetic relationships and selecting suitable genetic materials for breeding and improvement programs (1). Genetic diversity represents a cornerstone in wheat breeding and improvement programs, providing diverse genetic resources that can be utilized in selecting genetically distant parents and developing cultivars that combine high productivity with adaptability and genetic stability. However, repeated reliance on a limited number of superior cultivars has contributed to narrowing the genetic base of some modern breeding materials, potentially limiting their genetic flexibility and ability to respond to environmental variables. Modern genomic analyses have

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shown that improved cultivars have not fully captured the variations present in landraces, which still retain distinctive genetic variations and structures that have not yet been exploited and can be employed in genetic improvement programs. Therefore, characterizing local germplasm is a pivotal step for uncovering latent variation and identifying suitable structures for future selection and hybridization programs (2). DNA molecular markers are effective tools for detecting molecular polymorphism and estimating genetic relationships, as they rely on differences in genetic material and are not directly influenced by environmental conditions or plant growth stage. The Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) technique is characterized by ease of application, speed, and no requirement for prior genomic sequence information. The technique uses arbitrary short primers to amplify multiple unknown genomic loci. Differences in primer binding sites or changes in the distances between them result in polymorphic bands among genetic constitutions (3). The banding patterns generated by the RAPD-PCR technique are converted into a binary data matrix, where band presence is coded as 1 and absence as 0. These data are used to calculate genetic similarity and genetic distance coefficients, as well as to clarify relationships among genetic constitutions through cluster analysis and constructing a dendrogram using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Furthermore, the total number of bands, polymorphic bands, polymorphism percentage, primer efficiency, and discriminating power are used to evaluate the efficiency of primers and their ability to reveal molecular differences. Unique and absent bands are considered diagnostic markers contributing to the identification of DNA fingerprints for some studied constitutions. Studies by (3) and (4) demonstrated the efficiency of RAPD-PCR markers in detecting polymorphism, estimating genetic relationships, and identifying distinctive bands for certain genotypes. Locally, a study by (5) revealed genetic variation among several Iraqi durum wheat cultivars using RAPD, ISSR, and CDDP molecular markers. Cluster analysis also helped clarify genetic relationships and classify cultivars into groups reflecting varying degrees of genetic relatedness. In the same context, (4) showed variation in RAPD banding patterns among bread wheat genotypes grown in Iraq, in addition to the appearance of unique bands and variation in genetic similarity values. These results indicate the molecular variation possessed by Iraqi wheat cultivars, which can be utilized in breeding and genetic improvement programs, and also highlight the need to expand studies to include cultivated genotypes in different Iraqi regions, especially local genotypes whose molecular characterization data are still limited. This direction is supported by the results of (8), where RAPD markers distinguished 16 Iraqi cultivars and revealed 55 polymorphic bands. Similarly, (9) used six RAPD primers to characterize 17 Iraqi genotypes, reporting 365 amplification loci, and cluster analysis divided the genotypes into two main groups. A recent Iraqi study using ISSR markers also found clear molecular variation among ten cultivars and their distribution into two main groups via the UPGMA method (10). These local results provide a suitable comparative framework for interpreting genetic variation and fingerprinting in the cultivars of the current

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study. Based on this, the present study aimed to assess the level of genetic diversity, determine relationships, and establish genetic fingerprints for eleven local wheat genotypes cultivated in Salah al-Din Governorate using RAPD-PCR markers. This was achieved by analyzing banding patterns, estimating polymorphism percentages and primer efficiencies and discriminatory power, identifying unique and absent bands, calculating similarity and genetic distance coefficients, and illustrating the pattern of genetic relatedness and divergence among the studied genotypes using cluster analysis with the UPGMA method. The results are expected to provide a molecular information base that contributes to distinguishing the studied genotypes, determining their genetic proximity or divergence, and assessing their potential utility in genetic resource conservation and wheat improvement programs.

Materials and Methods

Molecular Study

The molecular study was conducted at the Molecular Biology Laboratory, Department of Life Sciences, College of Science, University of Tikrit. It included the collection of plant samples and the execution of necessary molecular analyses to study the genetic variation among the studied local wheat genotypes using the RAPD-PCR technique. This section presents the steps for sample collection, genomic DNA extraction, concentration and purity estimation, detection by electrophoresis, RAPD-PCR reactions, and analysis of the resulting banding patterns.

Samples Collection

Leaf samples were collected from the eleven wheat genotypes 15 days after planting. Young leaves were taken from field-grown plants on 1/12/2025. The samples were then transferred to the Molecular Biology Laboratory / Department of Biology / College of Science / University of Tikrit for DNA extraction and subsequent molecular analyses for the RAPD-PCR technique.

Genomic DNA Extraction

Genomic DNA was extracted from the young leaves of the eleven wheat genotypes. Tissue portions were cut using sterile scissors and transferred to a clean ceramic mortar, where they were ground with liquid nitrogen until a fine powder was obtained. The powder was transferred to 2 ml Eppendorf tubes, and 800 µl of CTAB solution was added. Samples were mixed using a Vortex for one minute. The samples were incubated in a water bath at 65°C for 25–30 minutes with mixing every 5 minutes, then left at room temperature for 10 minutes. Then, 600 µl of chloroform was added, and the mixture was vortexed for one minute. After that, centrifugation was performed at 13,000 rpm for 5 minutes, and 500–600 µl of the supernatant was transferred to a new tube. Then, 600 µl of Binding Buffer was added. The mixture was transferred to a spin column and centrifuged at 11,000 rpm for 1 minute. The column was then washed sequentially with 500 µl of Washing Buffer 1 and 500 µl of Washing Buffer 2, with centrifugation after each wash. An additional centrifugation step at 13,000 rpm for 3 minutes was performed to remove residual ethanol.

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Finally, the spin column was transferred to a new 1.5 ml Eppendorf tube, and 100 µl of pre-heated (65°C) DNA Elution solution was added. It was left for 5 minutes at room temperature, then centrifuged at 11,000 rpm for 2 minutes. The column was discarded, and the eluate containing the extracted DNA was preserved for subsequent molecular analyses.

Measuring DNA Concentration and Purity Using NanoDrop The concentration and purity of the genomic DNA extracted from wheat samples were measured using a NanoDrop spectrophotometer, based on absorbance readings at 260 nm and 280 nm wavelengths. The device was calibrated using the elution buffer used for the samples. Then, 1 µl of the extracted DNA solution was placed on the measurement pedestal, and the DNA concentration and A260/A280 ratio were recorded to assess sample purity before use in RAPD-PCR reactions.

Detection of DNA by Agarose Gel Electrophoresis

The quality of the DNA extracted from wheat samples was verified using agarose gel electrophoresis. A 1% agarose gel was prepared by dissolving agarose powder in 1X SB buffer. The solution was allowed to cool to approximately 55°C, and 1.5 µl of RedSafe stain was added. The gel was then poured into the casting tray and left to solidify. After solidification, the gel was placed in an electrophoresis tank containing 1X SB buffer. A mixture of 5 µl of the DNA sample and 2 µl of Loading Buffer was loaded into the gel wells. Electrophoresis was performed at a voltage of 3 V/cm across the gel for 50–60 minutes. The gel was then examined under a UV Transilluminator at 302 nm wavelength to visualize and photograph DNA bands.

RAPD-PCR Reactions

RAPD-PCR (Random Amplified Polymorphic DNA-PCR) reactions were performed on the DNA extracted from the eleven wheat genotypes, based on the method described by (12). A total of 21 random primers (Operon Technologies, USA) were used in the amplification process to detect variation in banding patterns and estimate the level of genetic diversity among the studied genotypes.

Primers Used in RAPD-PCR

The random primers used in the RAPD-PCR reactions and their nucleotide sequences are listed below:

Table(1) The actual list of primer sequences from the original text was not included in the provided file content snippet after this point.

Sequence (5′→3′)	Primer Code	No.
GGTGACGCAG	OPB-07	P1
GGGGGTCTTT	OPC-03	P2
CCGCATCTAC	OPC-04	P3
GATGACCGCC	OPC-05	P4
CTCACCGTCC	OPC-09	P5
TGTCTGGGTG	OPC-10	P6
AGCGCCATTG	OPD-11	P7

ISSN 2072-3857

ACCGGCTCAC	OPD-20	P8
CAGGCCCTTC	OPA-01	P9
TGCCGAGCTG	OPA-02	P10
AATCGGGCTG	OPA-04	P11
CTACCCCCTG	OPB-03	P12
TTCGAGCCAG	OPF-07	P13
TTGGCACGGG	OPD-07	P14
GGCAGGCTGT	OPE-08	P15
CCTGATCACC	OPF-03	P16
AGCGTGTCTG	OPG-04	P17
GAATCGGCCA	OPH-18	P18
CAGCGACAAG	OPI-07	P19
CCACATCGGT	OPU-09	P20
GGACTGGAGT	OPB-04	P21

Preparation of RAPD-PCR Reaction Mixture

The RAPD-PCR reaction mixture was prepared in a final volume of 20 µL per sample. It consisted of 10 µL of Prime Taq Premix (Master Mix), 2 µL of the random primer, 2 µL of DNA template, and 6 µL of distilled water. The reaction components were mixed well, then distributed into the reaction tubes of the thermal cycler in preparation for the amplification process.

Volume (µL)	**contain**
10	Prime Taq Premix (Master Mix)
2	Primer
2	DNA Template
6	Nuclease-Free Water
20	Final Volume

RAPD-PCR Amplification Conditions

The amplification reactions were performed using a PCR Thermal Cycler according to a thermal program that included an initial denaturation at 95°C for 4 minutes, followed by 40 cycles. Each cycle consisted of denaturation at 94°C for 50 seconds, primer annealing at 36°C for 1 minute, and extension at 72°C for 1 minute. A final extension step was then performed at 72°C for 10 minutes.

Stage	**Temperature**	**Temperature**	**Duration**	**Number of Cycles**
Initial denaturation of the DNA strand	**95°C**	**4 min.**		**1**
Complete denaturation of the double-stranded DNA	**94°C**	**50 sec.**		**40**

ISSN 2072-3857

Primer annealing	**36°C**	**1 min.**	
Elongation / Extension	**72°C**	**1 min.**	
Final elongation / Final extension	**72°C**	**10 in.**	**1**

Agarose Gel Preparation

A 2% (w/v) agarose gel was prepared for electrophoresis of the RAPD-PCR products. This was done by dissolving 1 g of agarose powder in 50 mL of 1X SB buffer. The mixture was heated until the agarose completely dissolved and the solution became homogeneous and clear. It was then allowed to cool to a suitable temperature, poured into the electrophoresis casting tray, and the comb was inserted to form wells for sample loading. The gel was left to solidify completely.

Electrophoresis of RAPD-PCR Products

After the agarose gel had solidified, it was placed in the electrophoresis tank containing 1X SB buffer. Then, 4 µL of each RAPD-PCR reaction product was loaded into the designated wells. Additionally, 5 µL of DNA ladder (molecular weight marker) was loaded into the first well to estimate the molecular sizes of the resulting bands. Electrophoresis was performed at a voltage of 75 volts for 50–60 minutes. After electrophoresis, the gel was transferred to a Gel Documentation System for visualization and photography of the DNA bands.

Visualization and Documentation of RAPD-PCR Products

After the electrophoresis run was completed, the agarose gel was transferred to a Gel Documentation System equipped with a UV transilluminator. The gel was exposed to ultraviolet light at a wavelength of 302 nm to visualize the DNA bands resulting from the amplification process. The bands were then photographed, and the images were saved for subsequent analysis of banding patterns and estimation of molecular sizes by comparison with the DNA ladder.

RAPD Data Analysis

The banding patterns generated by the RAPD-PCR primers were analyzed based on the presence or absence of bands. The presence of a band was recorded as 1 and its absence as 0 for each of the eleven studied genotypes. The data were organized in the form of a binary matrix. The molecular sizes of the bands were estimated by comparison with the DNA (ladder). The resulting data were used to estimate variation indices and genetic relationships among the studied genotypes.

Calculation of RAPD Primer Indices

The percentage of polymorphism, primer efficiency, and discriminating power were calculated for each of the RAPD-PCR primers according to the following equations:

Polymorphism Percentage (%):

(The equation was not fully included in the provided text, but it typically is calculated as:)

ISSN 2072-3857

Polymorphism %= Number of Polymorphic Bands
Total Number of Bands×100
Polymorphism %= Total Number of Bands
Number of Polymorphic Bands ×100
(If the original text included additional equations for primer efficiency and discriminating power, they would be translated similarly; however, the provided snippet ends with the polymorphism percentage heading.)
If you actually need the translation back into Arabic (which it already is), please clarify. Otherwise, I hope this English translation meets your needs.

$$Variation\ \% = \frac{Np}{Nt} \times 100$$

Where:

- **Np**: Number of polymorphic loci produced by the primer.
- **Nt**: Total number of loci produced by the same primer.

- Primer Efficiency (%):
(No formula was provided in your text; please supply it if you need translation or calculation.)

$$Primer\ Efficiency = \frac{\text{TBp}}{\Sigma\text{TB}} \times 100$$

Where:
TBp: The total number of bands produced by the primer.
ΣTB: The total number of bands produced by all primers.
-Discriminatory Power of the Primer:(%)

$$\text{Discriminating Power} = \frac{\text{PLp}}{\Sigma\text{PL}} \times 100$$

Where:
PLp: The number of polymorphic loci produced by the primer.
ΣPL: The total number of polymorphic loci produced by all primers.

Genetic Relationships and Cluster Analysis

The analysis of genetic relationships among the eleven studied genotypes relied on the band data generated by the RAPD-PCR primers after converting them into a binary matrix. Band presence was recorded as 1 and absence as 0. These data were used to estimate the genetic distances between the studied genotypes using the NTSYS-pc software according to (12) (NEI72). Subsequently, cluster analysis was performed, and a dendrogram was constructed using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method to clarify the genetic relationships and the degree of relatedness and divergence among the studied genotypes, classifying them into groups based on the molecular band patterns generated by the RAPD-PCR markers.

ISSN 2072-3857

Results and Discussion

Results of RAPD-PCR Primers

All 21 random primers succeeded in producing scorable amplification bands in the eleven wheat cultivars. The band patterns varied in terms of locus number, sizes, and distribution among the cultivars, indicating differences in primer binding sites within the studied genomes and the presence of genetic variation that can be relied upon to distinguish cultivars and estimate their relationships. The primers produced 169 loci, of which 45 were monomorphic and 124 were polymorphic, giving a total polymorphism percentage of 73.37%. The total number of bands was 1300, comprising 495 monomorphic bands, 805 polymorphic bands, in addition to 13 unique bands and 32 absent bands. The primers OPB-07, OPC-04, and OPD-07 recorded the highest number of loci (11 loci each), while primers OPA-01 and OPA-04 recorded the lowest number (four loci each). OPC-04 produced the highest total number of bands (108 bands), whereas OPA-04 recorded the lowest (15 bands).

Results of loci and bands produced by the used primers

No.	Primer	Total Loci	Monomorphic Loci	Polymorphic Loci	Total Bands	Monomorphic Bands	Polymorphic Bands
1	03-OPB	8	1	7	60	11	49
2	01-OPA	4	2	2	38	22	16
3	02-OPA	7	3	4	49	33	16
4	07-OPB	11	4	7	77	44	33
5	07-OPI	10	1	9	92	11	81
6	09-OPC	10	7	3	105	77	28
7	20-OPD	9	1	8	71	11	60
8	03-OPC	8	4	4	70	44	26
9	04-OPC	11	4	7	108	44	64
10	05-OPC	9	4	5	60	44	16
11	03-OPF	6	0	6	42	0	42
12	04-OPG	6	1	5	57	11	46
13	04-OPA	4	0	4	15	0	15
14	04-OPB	9	2	7	67	22	45
15	10-OPC	10	0	10	72	0	72

ISSN 2072-3857

No.	Primer	Total Loci	Monomorphic Loci	Polymorphic Loci	Total Bands	Monomorphic Bands	Polymorphic Bands
16	07-OPD	11	2	9	49	22	27
17	11-OPD	6	1	5	48	11	37
18	08-OPE	9	5	4	80	55	25
19	07-OPF	6	0	6	29	0	29
20	18-OPH	9	1	8	70	11	59
21	09-OPU	6	2	4	41	22	19
Total	—	169	45	124	1300	495	805

مؤشرات كفاءة البوادئ والحزم الفريدة والغائبة

Primer	Unique Bands	Absent Bands	Molecular Size (bp)	Polymorphism (%)	Primer Efficiency (%)	Discriminatory Power (%
03-OPB	0	1	620–1700	87.50	4.62	5.64
01-OPA	0	0	400–1500	50.00	2.92	1.61
02-OPA	1	0	150–1700	57.14	3.77	3.22
07-OPB	2	1	250–1700	63.63	5.92	5.64
07-OPI	0	6	320–1600	90.00	7.07	7.25
09-OPC	0	1	450–1700	30.00	8.07	2.41
20-OPD	0	5	250–1800	88.88	5.46	6.45
03-OPC	0	1	350–1700	50.00	5.38	3.22
04-OPC	0	2	280–1600	63.63	8.30	5.64
05-OPC	1	0	150–1600	55.55	4.61	4.03
03-OPF	0	1	350–1300	100.00	3.23	4.83
04-OPG	0	3	480–1700	83.33	4.36	4.03
04-OPA	0	0	300–900	100.00	1.15	3.22
04-OPB	1	2	650–1800	77.77	5.15	5.64
10-OPC	1	2	500–1700	100.00	5.53	8.06
07-OPD	4	0	180–1300	81.81	3.76	7.25

ISSN 2072-3857

Primer	Unique Bands	Absent Bands	Molecular Size (bp)	Polymorphism (%)	Primer Efficiency (%)	Discriminatory Power (%
11-OPD	0	1	250–1700	83.33	3.69	4.03
08-OPE	0	1	200–1800	44.44	6.15	3.22
07-OPF	1	1	180–1400	100.00	2.23	4.83
18-OPH	1	4	320–1600	88.88	5.38	6.45
09-OPU	1	0	200–950	66.66	3.15	3.22
Total	13	32	1800–150	73.37	100.00	100.00

The polymorphism percentage reached 100% in primers OPF-03, OPA-04, OPC-10, and OPF-07, while OPC-09 recorded the lowest percentage at 30%. OPC-04 achieved the highest primer efficiency at 8.30%, whereas OPA-04 recorded the lowest efficiency at 1.15%. Regarding discriminatory power, the highest value was recorded for OPC-10 at 8.06%, and the lowest value was recorded for OPA-01 at 1.61%. The total band sizes ranged between 150 and 1800 bp, reflecting differences in the genomic regions targeted by the different primers. The total number of absent bands was 32. OPI-07 recorded the highest number of absent bands (six bands), followed by OPD-20 with five bands, then OPH-18 with four bands, and OPG-04 with three bands. The absence of a band indicates the failure to amplify the corresponding locus in a particular cultivar and may be associated with changes in the primer binding site sequence or the distance between the two binding sites. Therefore, the combined pattern of presence and absence provides a more reliable basis for estimating variation than relying on a single band. These results confirm the variation in discriminatory power among primers depending on the number of polymorphic loci they reveal. Furthermore, using a set of primers rather than relying on a single primer provides broader genome coverage and enhances the accuracy of cultivar characterization. This is consistent with the findings of (3) (4) regarding the efficiency of RAPD markers in detecting polymorphism and discriminating among bread wheat genotypes. When compared with RAPD studies on wheat, the total polymorphism percentage in the present study (73.37%) was higher than the 63.3% reported by (6) and the 43.39% reported by (7). However, this difference is not attributed solely to genetic variation but is also influenced by the number of genotypes and primers tested, as well as band scoring criteria. Therefore, the current percentage should be understood as evidence of the ability of the primer set used to detect high polymorphism within the studied local material. Furthermore, the study by (11), one of the most recent applications of RAPD in wheat, confirms the effectiveness of this marker in characterizing diversity among cultivars under salinity stress conditions.

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Genetic Fingerprinting

The results of the RAPD-PCR markers revealed the appearance of 13 unique bands that contributed to distinguishing six cultivars of local wheat within the studied group. The cultivar 'Dhahab' recorded the highest number of unique bands (five bands), four of which were produced by primer OPD-07 with molecular sizes of 680, 500, 250, and 180 bp, and one band produced by primer OPH-18 with a size of 900 bp. This was followed by the cultivar 'Mawaddah' with four unique bands: two appeared with primer OPB-07 (sizes 280 and 250 bp), one with primer OPC-05 (size 150 bp), and another with primer OPU-09 (size 300 bp). Additionally, each of the cultivars 'Qimah', 'Wafiyah', 'Sulaymaniyah', and 'Buhuth 22' recorded one unique band. The unique band for 'Qimah' appeared with primer OPA-02 (size 450 bp), for 'Wafiyah' with primer OPC-10 (size 550 bp), for 'Sulaymaniyah' with primer OPB-04 (size 1600 bp), while the unique band for 'Buhuth 22' appeared with primer OPF-07 (size 250 bp). These bands represent specific diagnostic loci that can be utilized as molecular markers to distinguish these cultivars within the studied group and to establish their genetic fingerprints.

The table shows the unique bands identified for the genetic fingerprinting of local wheat cultivars using RAPD-PCR markers.

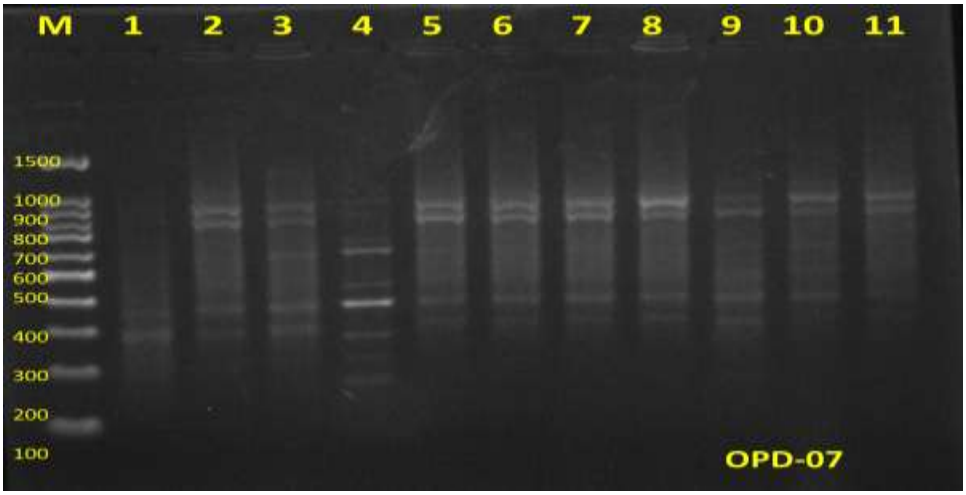
Cultivar	Primer	Unique Band Sizes (bp)	Number of Unique Bands
Mawaddah	OPB-07	250 ،280	2
Mawaddah	OPC-05	150	1
Mawaddah	OPU-09	300	1
Buhuth 22	OPF-07	250	1
Dhahab	OPD-07	180 ،250 ،500 ،680	4
Dhahab	OPH-18	900	1
Qimah	OPA-02	450	1
Wafiyah	OPC-10	550	1
Sulaymaniyah	OPB-04	1600	1
Total	—	—	**13**

Note: The cultivars 'Sham', 'Fayyad', 'Ibaa 99', 'Holir', and 'Babil' did not record any unique bands with the primers used. However, they were distinguished from one

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another based on the overall pattern of presence and absence of polymorphic bands.

For the purposes of concise presentation in the research, primers OPD-07 and OPB-07 were selected as representative examples because they contributed the highest number of unique bands in the cultivars 'Dhahab' and 'Mawaddah', respectively. Meanwhile, the numerical results for all primers were included in the two preceding tables .

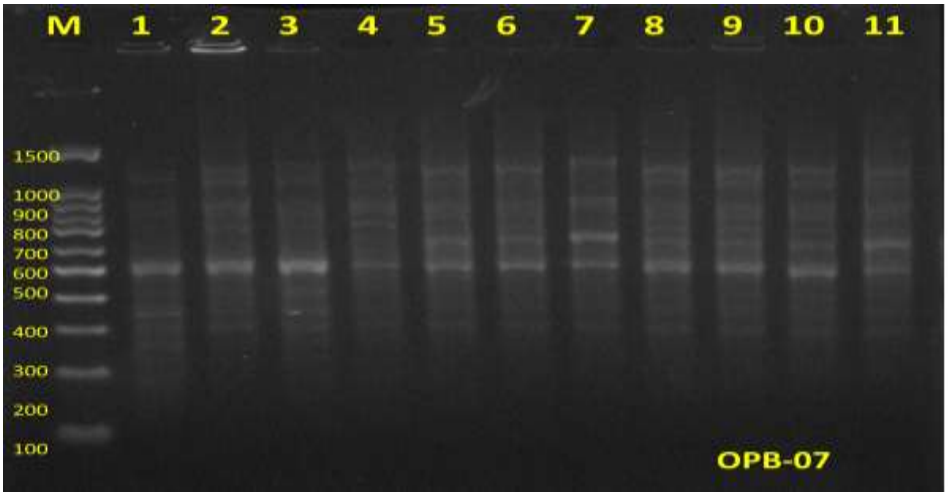


The figure shows the DNA banding pattern obtained from amplifying the eleven wheat genotypes using primer OPD-07 with the RAPD-PCR technique. M represents the DNA molecular weight marker, and numbers (1–11) represent the studied genotypes.

OPD-07	0	1	0	0	1	0	0	0	1	1	1	1300bp
	1	1	1	1	1	1	1	1	1	1	1	1000bp
	0	1	1	0	1	1	1	1	1	1	1	850bp
	0	0	0	1	0	0	0	0	0	0	0	680bp
	0	0	0	1	1	0	0	0	0	1	0	650bp
	0	0	0	1	0	0	0	0	0	0	0	500bp
	1	1	1	1	1	1	1	1	1	1	1	400bp
	0	0	0	0	1	1	1	1	1	0	0	380bp
	1	1	1	0	0	0	0	0	0	0	0	300bp
	0	0	0	1	0	0	0	0	0	0	0	250bp
	0	0	0	1	0	0	0	0	0	0	0	180bp

The table shows the molecular banding pattern produced by primer OPD-07 in the eleven wheat genotypes, where the number (1) indicates the presence of a band and the number (0) indicates its absence.

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The figure shows the DNA banding pattern obtained from amplifying the eleven wheat genotypes using primer OPB-07 with the RAPD-PCR technique. M represents the DNA molecular weight marker, and numbers (1–11) represent the studied genotypes.

OPB-07												
	0	0	0	0	0	1	1	0	0	0	0	1700bp
	0	1	1	1	1	1	1	1	1	1	1	1300bp
	1	1	1	1	0	0	0	1	1	1	1	1200bp
	1	1	1	1	1	1	1	1	1	1	1	850bp
	0	0	0	1	1	1	1	1	1	1	1	650bp
	1	1	1	1	1	1	1	1	1	1	1	500bp
	1	1	1	0	0	0	0	0	0	0	0	400bp
	1	1	1	1	1	1	1	1	1	1	1	350bp
	1	1	1	1	1	1	1	1	1	1	1	300bp
	1	0	0	0	0	0	0	0	0	0	0	280bp
	1	0	0	0	0	0	0	0	0	0	0	250bp

The table shows the molecular banding pattern produced by primer OPB-07 in the eleven wheat genotypes, where the number (1) indicates the presence of a band and the number (0) indicates its absence.

The appearance of unique bands reflects differences in the binding sites of random primers within the genomes of the studied cultivars, resulting from DNA sequence variations that may prevent amplification in some cultivars while allowing it in a specific cultivar, thus generating a distinctive molecular pattern for it. The recording of nine unique bands in the cultivars 'Dhahab' and 'Mawaddah' indicates that they possess the highest molecular discriminatory ability within the studied cultivar group, while the single unique bands contributed to distinguishing the cultivars 'Qimah', 'Wafiyah', 'Sulaymaniyah', and 'Buhuth 22'. The absence of unique bands in the remaining cultivars does not imply their genetic identity, as they can still be

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distinguished based on the overall pattern of polymorphic band presence and absence, as well as genetic distance values among them. This finding is consistent with the results of (4), in which RAPD primers revealed unique bands of different molecular sizes that contributed to identifying molecular fingerprints for some bread wheat genotypes and distinguishing them from the rest. Thus, the current results confirm the efficiency of unique bands in characterizing cultivars and establishing their genetic fingerprints, while their diagnostic significance is limited to the cultivars, primers, and amplification conditions used in the present study.

The appearance of unique bands as diagnostic markers is consistent with the findings of (6), as their study recorded seven unique bands when analyzing 36 wheat genotypes using RAPD. This further supports the use of unique bands in preliminary discrimination among genotypes, provided they are interpreted alongside the overall presence/absence pattern and their stability is verified through repetition.

Estimation of Genetic Relationships and Cluster Analysis

Genetic Relationships and Cluster Analysis

The band data generated by the RAPD-PCR primers, recorded as a binary matrix where band presence was coded as 1 and absence as 0, were used to estimate the genetic relationships among the eleven local wheat cultivars. Similarity coefficients and genetic distances were calculated, and the results were used to construct a dendrogram using the UPGMA method to reveal the levels of genetic relatedness and divergence among the cultivars.

Genetic Distance

The genetic distance matrix calculated according to (12) revealed variation in the degree of genetic relatedness among the eleven local wheat cultivars. Genetic distance values ranged between 0.019 and 0.424. The lowest genetic distance value (0.019) was recorded between the cultivars 'Fayyad' and 'Ibaa 99', indicating a high degree of genetic relatedness between them compared to other pairs. Conversely, the cultivars 'Sulaymaniyah' and 'Mawaddah' recorded the highest genetic distance value (0.424), reflecting the greatest degree of genetic divergence within the studied cultivar group.

Other genetic relationships were also observed, most notably the relatedness between 'Qimah' and 'Ibaa 99' with a genetic distance value of 0.044, and between 'Qimah' with both 'Fayyad', 'Holir', and 'Wafiyah' with a value of 0.048 for each, as well as the relatedness between 'Fayyad' and 'Holir' with a value of 0.055. The matrix also showed relative genetic relatedness between 'Sham' and 'Buhuth 22', with a genetic distance value of 0.186, indicating that they share part of their genetic background while each retains a degree of genetic difference. These low values suggest that these cultivars share a high proportion of the molecular loci revealed by the RAPD primers, which may be attributed to the relatedness of their genetic origins or shared parental lines used in breeding and improvement programs.

The high genetic distance value between 'Mawaddah' and 'Sulaymaniyah' reflects increased differences in the presence/absence banding patterns

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between them, resulting from variations in RAPD primer binding sites or sequence changes at those sites such as substitutions, deletions, or insertions. In general, high genetic distance values indicate low molecular similarity between genotypes, while low values reflect their relatedness in a greater number of recorded loci. This is consistent with what (3) reported on local bread wheat genotypes, attributing this to differences in genetic backgrounds and banding patterns

The current results are also consistent—in terms of exhibiting varying levels of relatedness and divergence among Iraqi wheat cultivars—with previous local studies that used RAPD or similar molecular markers. The study by (8) revealed genetic variation among Iraqi cultivars, and studies by (9), (5), and (10) demonstrated a clear population structure among wheat cultivars. This confirms that cultivars grown within the same geographical environment are not necessarily genetically identical due to differences in their origins, sources, and the selection programs they have undergone.

The genetic distance matrix indicates that the studied wheat cultivars do not represent a completely genetically homogeneous group; rather, they include highly related cultivars such as 'Fayyad' and 'Ibaa 99', and clearly divergent ones, particularly 'Mawaddah' and 'Sulaymaniyah'. This variation enhances the importance of the studied genetic material in characterizing cultivars and establishing their molecular fingerprints, and also provides preliminary information that can be utilized in selecting suitable genetic constitutions for breeding and improvement programs.

Let me know if you need the actual matrix table translated or any other sections.

Sulaymaniyah	Babil	Wafiyah	Holir	Ibaa 99	Fayyad	Qimah	Dhahab	Buhuth 22	Sham	Mawaddah	
										0.000	Mawaddah
									0.000	0.308	Sham
								0.000	0.186	0.186	Buhuth 22
							0.000	0.227	0.306	0.396	Dhahab
						0.000	0.211	0.222	0.192	0.358	Qimah
					0.000	0.048	0.188	0.200	0.190	0.352	Fayyad
				0.000	0.019	0.044	0.182	0.204	0.194	0.356	Ibaa 99
			0.000	0.067	0.055	0.072	0.206	0.198	0.169	0.347	Holir
		0.000	0.048	0.103	0.082	0.100	0.214	0.204	0.175	0.350	Wafiyah
	0.000	0.115	0.149	0.174	0.170	0.153	0.221	0.242	0.242	0.378	Babil,

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0.000	0.190	0.128	0.123	0.147	0.153	0.145	0.263	0.274	0.230	0.424	Sulaymaniyah

Cluster Analysis

It is evident from the genetic relatedness dendrogram shown in the figure, based on RAPD marker data, that the studied wheat cultivars were distributed into two main groups, with secondary branches within the second group, according to their degree of genetic relatedness or divergence.

Main Group I (Cluster I):

This group was confined to the cultivar 'Mawaddah' , which appeared in an independent branch and separated from the rest of the cultivars at a value of approximately 0.34. Its isolated position and late joining to the other cultivars indicate that it possesses the highest level of genetic divergence among the studied wheat cultivars.

Main Group II (Cluster II):

This group included the remaining ten cultivars and was characterized by being larger in number and more branched, further dividing into several sub‑groups:

• The cultivar 'Dhahab' (4) appeared in an independent branch within the second main group and joined the rest of the group's cultivars at a value of approximately 0.22, indicating its relative genetic divergence from them.

• One sub‑group included the cultivars 'Sham' (2) and 'Buhuth 22' (3), which met in a common branch at a value close to 0.19, indicating genetic relatedness between them. This sub‑group then joined the rest of the second group at a value of approximately 0.21.

• Another sub‑group included the cultivars 'Qimah' (5), 'Fayyad' (6), 'Ibaa 99' (7), 'Holir' (8), 'Wafiyah' (9), 'Babil' (10), and 'Sulaymaniyah' (11), which in turn branched into several secondary clusters. The cultivars 'Fayyad' (6) and 'Ibaa 99' (7) recorded the closest genetic relationship among the studied cultivars, meeting at the lowest value of approximately 0.02, then 'Qimah' (5) joined them at a value close to 0.05.

• A second branch was formed by the cultivars 'Holir' (8) and 'Wafiyah' (9), which met at a value of approximately 0.05, indicating a high degree of genetic relatedness between them. This branch joined the group of 'Qimah', 'Fayyad', and 'Ibaa 99' at a value close to 0.08.

• The cultivar 'Sulaymaniyah' (11) joined this group at a value of approximately 0.14, and then 'Babil' (10) joined at avalue close to 0.16, forming, together with the previous cultivars, a broad sub‑group within the second main group.

This cluster distribution demonstrates the ability of RAPD markers to discriminate among the studied wheat cultivars and classify them into distinct genetic groups based on their degrees of relatedness and divergence.

The cluster analysis based on RAPD marker data revealed clear genetic variation among the studied wheat cultivars, as the cultivars were distributed into two main groups containing several sub‑clusters with varying degrees of genetic relatedness. This grouping pattern reflects differences in the molecular fingerprints

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of the cultivars resulting from differences in the binding sites and distribution of random primers across the genome, which led to variation in banding patterns and consequently differences in the genetic distance coefficients upon which the dendrogram was constructed.

The cultivar 'Mawaddah' appeared in a separate branch and joined the other cultivars at a relatively high divergence level of approximately 0.34, indicating that it possesses a more distinct genetic constitution compared to the rest of the studied cultivars. The presence of groups with varying degrees of genetic relatedness is consistent with the findings of (3), who classified bread wheat genotypes into three main groups using UPGMA cluster analysis based on RAPD and ISSR markers. The comparison here lies in revealing the variation in genetic relationships among the studied genotypes, with differences in the number of groups between the two studies. In the present study, cluster analysis using RAPD markers on wheat genotypes grown in Iraq isolated one genotype in a separate group while the other genotypes clustered together in a second group, reflecting the ability of RAPD markers to detect the most genetically divergent genotypes.

In contrast, some cultivars showed a high degree of genetic relatedness, most notably the cultivars 'Fayyad' and 'Ibaa 99', which met at the lowest value in the dendrogram (approximately 0.02). This result is consistent with the low genetic distance value recorded between them in the genetic distance matrix. This relatedness can be explained by a significant degree of similarity in the genomic loci amplified by the RAPD primers, which may reflect similarity in the genetic background of these two cultivars. Previous studies have reported that the clustering of some wheat cultivars in closely related branches in a UPGMA dendrogram is associated with high similarity in molecular banding patterns (4).

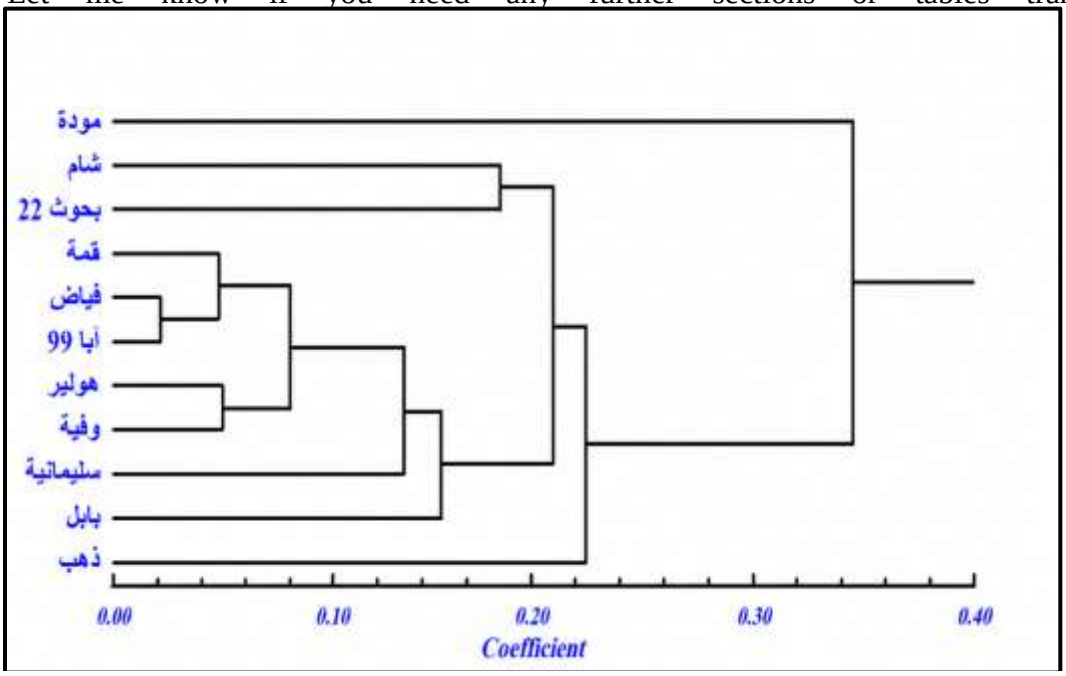
Likewise, the clustering of 'Qimah' with 'Fayyad' and 'Ibaa 99', and the relatedness of 'Holir' with 'Wafiyah', followed by the joining of these branches into one sub‑group, confirms that the genetic relationships among the cultivars were not uniform but ranged from very close relatedness between some cultivars to relative divergence between others. This is supported by the findings of (3).

The division of the cultivars in the present study into two main groups further confirms the ability of absent and unique band markers to reveal population structure. This overall pattern is consistent with the results reported by. (9) (7), and (10), which divided wheat genotypes into two main groups, albeit with differences in genetic material, marker type, and number of primers among the studies.

Accordingly, the dendrogram summarizes the pattern of genetic relationships derived from the genetic distance matrix, as cultivars with low genetic distances clustered into closely related branches, while more divergent cultivars joined the rest of the groups at higher levels. These results indicate the potential utility of RAPD markers in characterizing genetic diversity and discriminating among the studied wheat cultivars within the locations covered by the analysis. Furthermore, the genetic divergence information can contribute to guiding parental selection for breeding and genetic improvement programs,

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when combined with the evaluation of their phenotypic and agronomic traits. Let me know if you need any further sections or tables translated.



(Cluster analysis dendrogram based on genetic distance values of the studied local wheat genotypes)

Conclusions

The RAPD-PCR markers revealed clear molecular variation among the eleven local wheat cultivars, with a polymorphic loci percentage of 73.37%, and 13 unique bands and 32 absent bands were detected. The cultivars 'Dhahab' and 'Mawaddah' were distinguished by having the highest number of unique bands, supporting the potential use of these bands in establishing diagnostic molecular fingerprints within the limits of the cultivars, primers, and amplification conditions used. The genetic distance matrix also showed that 'Fayyad' and 'Ibaa 99' were the most closely related, while 'Mawaddah' and 'Sulaymaniyah' were the most divergent. The dendrogram confirmed the distinctness of the cultivar 'Mawaddah' in a separate group. These results provide an initial basis that can be utilized in the conservation of genetic resources and the selection of genetically divergent genotypes for breeding and improvement programs, with the necessity of supplementing the molecular selection with phenotypic and agronomic evaluation.

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