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Original Research Article

Determining the Genetic Fingerprinting and Genetic Distance of Bread Wheat varieties (*Triticum aestivum*) Using RAPD-PCR Markers

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Abstract: ***Objective***: The aim of the current search is to determine the DNA fingerprinting and genetic distance of eight varieties (Sham6, Sham9, Ibaa99, Fayyad, bohoth22, Nizar, Buro, Nazar) of local bread wheat grown in Tikrit city using RAPD -PCR Markers. ***Materials and Methods of Work***: DNA was extracted from the young leaves of the samples after (15) days of cultivation. The concentration and purity of the DNA were measured, and RAPD-PCR indicators were applied to the DNA of the eight varieties using (23) primers. The results sample were transferred on electrophoresis, exposed to ultraviolet light The gel was photographed, and the images were transferred for statistical analysis. ***Results***: The primers used produced (591) band, of which (96) were general band and (495) were different band. A genetic fingerprint was established for most of the genotypes, characterized by a number of distinctive bands totaling (43), of which (30) were unique and (13) were absent. The Sham 9 G2 variety had the high number of unique bonds, reaching (7) bands, The Nizar G8 variety had the fewest, possessing only one unique band. As for the absent bands, Own the Bohoth 22G3 variant with the high number of bands, reaching (3) bands. In contrast, the Sham 6 G1 variety absent bands. The primers varied in size, resulting in band sizes ranging from 100 to 2500 bp. The genetic distance values ranged (0.275 - 0.664), where the lowest genetic distance was between the two varieties, Inaa 99 G3 and Al-Nizar G6, If the ratio appears as 0.275, a considered the greatest similarity between the two types included in the search. As for the highest genetic distance, it reached 0.664 between the two varieties, Buhouth 22 G5 and Al-Buro G7 the lowest similarity. ***Conclusion***: The varieties were characterized by high genetic variation among and distinctive (unique and absent) bands. The RAPD markers showed high efficiency in determining the genetic fingerprint and genetic distance of the studied genetic structures.

Keywords: Fingerprinting, RAPD-PCR, *Triticum Aestivum, Genetic Distance*.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important and widely cultivated agricultural and economic crops in the world, primarily used for human and animal consumption, feeding approximately 35% of the world's population. The wheat constitutes about 95% of the total wheat currently grown globally and is used in the preparation of bread, pastries, and other baked goods or food (AL-Tamimi, 2014: Hameed *et al*., 2023). Given that wheat is a staple food in Iraq, identifying high-yielding varieties is a primary objective for wheat breeders in the country (Khodadadi *et al*., 2011). Using phenotypic traits is costly and requires considerable effort and time to establish a varietal fingerprint and determine genetic variation, whereas morphological differences may be epigenetic or genetic (Kumar *et al*., 2009).

The development of molecular markers (DNA) has provided a new precise method and completeness in examining the genetic resources of agricultural crops (Ayoob, 2020). Advances in molecular genetics in wheat are less advanced than in other cropshis is especially true when compared to some crops such as barley, tomatoes, and rice. This is mainly due to the high level of polyploidy in wheat, the large size and density of the genome, the very high proportion of tandem sequences, and the low level of genetic variation (Rana *et al.,* 2013). Assessing the diversity of genetic resources can help identify local varieties with high variability, and thus be the breeders in crop improvement programs (Shirmohammadi *et al*., 2018). Studying the genetic variation is important because it one of the objectives upon which plant breeders rely on to evaluate varieties in every breeding program, whether through selection, hybridization. It is also crucial for determining

quantitative and qualitative traits and for conserving genetic resources. Furthermore, it does not require prior knowledge of the nature of trait distribution and mirrors the field evaluation of varieties through laboratory analysis using polymorphic random amplification indices (Al-Rufaye, 2018).

A key step in improving field crops, including bread wheat, is the complete molecular characterization of their genetic resources. This can contribute to identifying genetic relationships and genetic diversity among breeding lines, enabling breeders to improve field crops (Al-Ghufaili and Al-Tamimi, 2018). The genetic diversity of plants determines their potential for improvement and future breeding, thus promoting high yields and self-sufficiency (Khodadadi *et al*., 2011). Efficient and rapid screening of these genotypes accelerates the variety evaluation process, and molecular indices play a pivotal role in this regard. Molecular indices are stable markers within the genome. In this study, random polymorphic DNA amplification (RAPD) (Williams *et al.,* 1990) was used to determine the genetic fingerprint and phylogenetic profile of six local wheat varieties cultivated in Tikrit to examine the genetic similarity/difference between certain wheat genetic origins (Kumar *et al*., 2017). In order to preserve the local varieties approved by the General Seed Company, this study aims to establish a genetic fingerprint for each local variety and some introduced varieties to protect them from being replaced by phenotypically similar varieties. The genetic fingerprinting was determined using indicators of the RAPD-PCR.

MATERIALS AND METHODS

Experimental Design

Wheat seeds were collected from the General Company for Seed Certification in Tikrit, consisting of five local varieties and three imported varieties approved for bread wheat cultivation in Iraq, as shown in Table (1). One month after the planting date for all varieties, (4-5) immature leaves were picked from the growth tip and placed in sterile, sorted bags, then brought directly to the laboratory to perform the DNA isolation process.

Table 1: Wheat varieties, their symbols used, and the supplying company

Symbol	Cultivar Name	The company that supplies the items
G1	Sham 6	Seed Testing and Certification Department/Accredited
G2	Sham 9	Seed Testing and Certification Department/Accredited
G3	Ibaa 99	Seed Testing and Certification Department/Accredited
G4	Fayyad	Seed Testing and Certification Department/Accredited
G5	bohoth 22	Seed Testing and Certification Department/Accredited
G6	Nizar	Seed Testing and Certification Department/Non-Accredited
G7	Buro	Seed Testing and Certification Department/Non-Accredited
G8	Nazar	Seed Testing and Certification Department/Non-Accredited

Extraction DNA and Purification DNA:

NA was obtained from the growing tips of wheat plants using the CTAB method as described (Weigand *et al*., 1993; Huang *et al*., 2013). DNA purification was performed according to the method described above (Al-Sugmany, 2017).

Concentration and Purity DNA Assessment:

DNA purity and concentration were assessed using a Nanodrup device. Sample concentrations were adjusted and prepared to a concentration of 50 ng/µL, then stored in the freezer until use.

Gel Electrophoresis:

The materials, electrophoresis solution, gel, and samples were prepared for electrophoresis according to the methods (Doyle and Doyle (1987) and Al-Sugmany, 2017).

Table 2: Sequence of primers used on wheat plants

NO	Primers name	S equence 5´→ → → 3´	number	Primers name	S equence 5´→ → → 3´
1	OPP -0 1	GTAGCA CTCC	11	OPJ -12	GT CC CG TGGT
2	OPG- 15	ACTGGG ACTC	12	OPJ - 14	CACCC GGATG
3	OPO- 11	GACAGG AGGT	13	OPG - 11	TGCCC GTCGT
4	OPB- 15	GGAGGG TGTT	14	OPQ - 15	GGGTA ACGTG
5	OPB- 12	CCTTGAC GCA	15	OPB - 20	GGACC CTTAC
6	0PA- 06	GGTCCCT GAC	16	OPB - 10	CTGCTG GGAC
7	OPP- 07	GTCCATG CCA	17	OPH- 01	GGTCGG AGAA
8	OPG - 13	CTCTCCG CCA	18	OPA-12	CCTGTAC CGA
9	OPD - 08	GTGTGCC CCA	19	OPH -08	GAAACAC CCC
10	OPD - 03	GTCGCCG TCA	20	OPN- 10	ACAACTG GGG

Performing RAPD-PCR Reactions:

RAPD-PCR reactions was performed on the eight bread wheat varieties using (20) randomly selected primers, following the method described with some modifications, as shown in Table 2. The main reaction mixture was prepared by combining the components in a sterile 1.5 mL Eppendorf tube. The mixture was then centrifuged for 3–5 seconds to ensure homogeneity (Qadir *et al*., 2015). The experiment was performed in a sterile hood, and the tubes were placed on a cooling rack, as shown in Table 3 below.

Table 3: Reaction components used in RAPD

No.	Components	Volume
1	Green Master mix	10 µ l
2	Primer	2 µ l
3	Nuclease free water	6 µ l
4	DNA Template	2 µ l
5	Total Volume	20µ l

Method of Work:

The RAPD-PCR reaction procedure shown in Table (4) was applied. After Reaction time was completed, Tubes were removed from the thermopolymer and kept frozen. (4) microliters were withdrawn from the tubes and then the mixture was placed in the wells of a pre-prepared agarose gel at a concentration of 1.5% with the volumetric indicator Ladder Marker. The samples were then transferred on an electrophoresis device at a voltage of 1.5 V/cm2 of the gel for 60 minutes. After the transfer was completed, the gel was exposed to the ultraviolet source on a UV-transilluminator and the images were taken using a high-resolution camera. The images were saved and entered into the software for statistical analysis.

PCR-RAPD Markers:

The reaction was carried out according to the following steps: First, an initial denaturation of one cycle at 95 °C for 4 minutes, then denaturation of 40 cycles at 93 °C for 50 seconds, primer annealing at 36 °C for one minute, elongation at 72 °C and 40 cycles for 1.5 minutes, and finally a final elongation of one cycle at 72 °C for 10 minutes. After completing the amplification in the PCR device, 5 microliters of the PCR-RAPD products are taken and transferred to a 1.5% agarose gel with DNA Marker, after which the gel is imaged using ultraviolet (UV) rays (Ezekiel *et al*., 2011).

Statistical Analysis:

a) Estimating the efficiency and discriminatory the efficiency of each primer was estimated using the equation mentioned in [13]: Efficiency = (Number of bands per primer / Total number of duplication bands for all primers) × 100

The discriminatory power was found based on the following equation: Discriminatory power = (Number of different bands per primer / Total number of different bands for all primers) × 100.

Estimating the Genetic Distance:

The genetic distance between the genotypes used is estimated by transferring the gel electrophoresis results to descriptive tables, assigning (1) when a beam is present on the sample and (0) when the beam disappears. To determine the genetic relationship between the genotypes, statistical analyses were performed using the computer program described in (Rohlf, 1993). This program employs the equations mentioned therein. (Nei, and Li ,1979).

RESULTS

To study the genetic fingerprint and genetic profile of eight genotypes, RAPD markers (23) random primers were used. Of these, (20) primers out of 23 were identified locations on the genome and produced different bands that were detected on agarose gel. One of these primers, OPD-02, produced universal bands for all samples, while the others produced bands that were either universal or heterozygous. Three primers (OPH-04, OPN-11, and OPO-01) did not identify any location on the genome because they did not produce bands on agarose gel. The use of a large number of primers in RAPD markers indicates increased accuracy in determining genetic profile and the ability to detect a larger area of the genome, especially when 95% of the primers used produce bands, and the majority produce heterozygous bands.

Primer Results:

The results of the primers used, shown in Table (5), revealed different band patterns. The total number of sites identified by the primers on the sample genome was (140), with an average of (7) bands per primer. Of these, (14) were common sites, averaging (0.75) per primer, and (126) were heterogeneous sites, averaging (6.6) bands per primer. The primers OPW-09 and OPG-11 produce the high number of sites, reaching (11) sites. Primer OPD-02 produced the fewest sites, reaching two, as shown in Figures (1, 2, 3, 4). The greater the number of heterogeneous bands, The determination of the genetic dimension increases with the efficiency of the primers, and increases the likelihood of obtaining a genetic

fingerprint for the varieties included in the breeding program. This is because the bread wheat genome has high heterogeneity, which led to the appearance of a high number of heterogeneous bands, reaching 90% of the total bands.

It was found that the total number of bands produced from these sites, as shown in Table (5), was (591) bands, of which (96) were main bands and (495) were polymorphic bands. The OPG-01 primer produced the highest number of bands, with (47) bands, while the OPM-10 primer produced the lowest number, with (11) bands. The overall variation ratio of the produced primers was 92%.

Table 4: Results of RAPD-PCR markers on wheat

No.	Primer Number	Loci number	Monomorphic loci	Polymorphic loci	Bands number	Monomorphic bands number	Polymorphic band number	Unique bands	Absent bands	variation ratio %
1	OPC- 05	4	-	4	14	-	14	1	1	100
2	OPQ -15	8	-	8	30	-	30	3	1	100
3	OPG- 15	6	1	5	20	8	12	1	-	83
4	OPJ -12	8	-	8	25	-	25	2	-	100
5	OPJ -14	10	1	9	41	8	33	-	1	90
6	OPG -11	11	-	11	33	-	33	2	-	100
7	OPW -08	9	2	7	39	16	23	2	1	77
8	OPW-09	11	2	9	40	16	24	4	-	81
9	OPG -01	11	1	10	47	8	39	3	2	90
10	OPO -04	8	1	7	30	8	22	2	1	87
11	OPA- 11	4	1	3	24	8	16	-	1	75
12	OPE - 16	6	1	5	29	8	21	2	2	83
13	OPA- 13	4	-	4	14	-	14	1	-	100
14	OPE - 20	5	1	4	23	8	15	1	-	80
15	OPO - 06	7	-	7	25	-	25	1	-	100
16	OPO 12	8	-	8	34	-	34	1	1	100
17	OPM -10	3	1	2	11	8	3	1	-	66
18	OPD - 02	2	2	-	16	16	-	-	-	0
19	OPAB- 12	5	-	5	25	-	25	-	1	100
20	OPH- 01	10	-	10	30	-	30	3	-	100
Total		140	14	126	591	96	495	30	12	92%

Table 5: Represents the distinctive bands in determining the genetic fingerprint of the wheat plant

Primer Name	Size Molecular	Distinctive bundles in wheat varieties																competenc	Discerning ability, e
		G1		G2		G3		G4		G5		G6		G7		G8			
	bp	U nique	A bsent	U nique	A bsent	U nique	A bsent	U nique	A bsent	U nique	A bsent	U nique	A bsent	U nique	A bsent	U nique	A bsent		
OPC- 05	100-800	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	1.5	2.2
OPQ -15	450-1000	-	-	1	-	-	-	1	-	-	-	1	-	-	-	-	-	3.5	4.6
OPG- 15	300-1100	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	2.2	1.9
OPJ -12	475-1500	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	2.3	3.2
OPJ -14	425-1800	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	4.9	5.5
OPG -11	425-1600	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	3.9	5
OPW -08	175-950	1	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	4.7	3.4
OPW-09	200-2500	-	-	1	-	2	-	-	-	-	-	1	-	-	-	-	-	3.4	3.6
OPG -01	175-2250	-	-	1	-	-	1	-	1	-	-	1	-	-	1	-	-	5.7	6
OPO -04	175-1300	-	-	-	-	-	1	-	-	1	-	1	-	-	-	-	-	3.3	3.7
OPE -20	300-1000	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	2.8	2.2
OPA- 13	250-550	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1.6	2.1
OPA- 11	150-850	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	2.9	2.4
OPE -16	150-1100	-	-	2	1	-	-	-	-	-	-	-	-	-	-	-	1	3.4	3.1
OPO -06	250-950	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	1	2.9	3.7
OPD -02	600-1700	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.9	0

OPO 12	200-725	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	4.1	5.2
OPM -10	200-650	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	0.3
OPH-01	300-1300	-	-	1	-	1	-	1	-	-	-	-	-	-	-	-	-	3.6	4.5
OPAB-12	475-1400	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	4.8	3.6
المجموع		2	0	7	2	6	3	3	2	3	2	5	1	3	2	1	1		
43		3		9		9		5		5		6		5		2			

Some genotypes were characterized by distinctive bands unique bands and absent bonds, and showing in Table 6. Summation number of distinctive bands result from primers produced in Search was (43), of which (30) were unique and (13) were absent. The Sham 9 G2 variety had the high percentage of unique bonds, reaching (7) bands, while the Nizar G8 variety had the fewest, possessing only one unique band. As to the absent bands, the Buhouth 22 G3 variety was characterized by the highest number of these bands, reaching (3) bands. In contrast, the Sham 6 G1 variety did not possess any absent bands.

This opens up future possibilities not only for finding distinctive bundles for other genetic structures but also for linking such segments with other field or analytical traits to facilitate the work of The primers produced varied in the resulting band sizes shown in Table (6). The sizes ranged between (100 -2500bp), with the smallest molecular size (100 bp) in the OPC-05 primer, while the largest molecular size (2500bp) was for the OPW-09 primers. The efficiency of the primers in showing the variation between the studied genetic structures varied. The lowest efficiency was for the OPM-10 primer, which reached (1.2), while the highest efficiency was for the OPJ-14 primer, which reached (4.9), as shown in Figures (5, 6). As for the discriminatory ability of the primers, the primer OPJ-14 was distinguished by the highest discriminatory ability, which reached (5.5), while the primer OPD-02 had a small discriminatory ability because the primer did not show different bands. From the results of the RAPD above, it is clear that these primers have different efficiency, but most of these primers had a suitable efficiency. It is concluded from this that the efficiency of the primer may increase with the increase in the number of bands produced, meaning that the primer that is able to show 10 bands for a certain genetic structure, with molecular sizes ranging between (100-2500 bp), is able to scan (5000 bp) of the genome of that genetic structure.

Estimating Genetic Distance Based on RAPD Indices:

Genetic variation was estimated in the results of RAPD-PCR index interactions between the bread wheat genotypes included in the study, using the genetic software NTSYS_PC, version 2.10. This software relies on the presence of shared bands between each genotype and its analysis is based on the equation mentioned in (Nei, and Li ,1979).

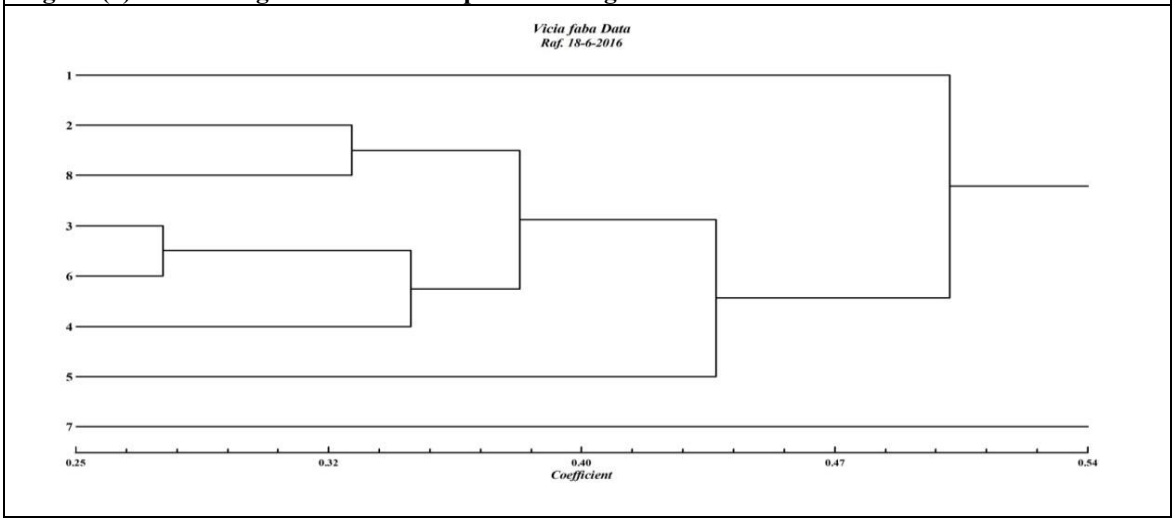
Table (7) shows the values of the genetic distances for the studied genotypes using (22) random RAPD primers. If the genomic material of two genotype is identical, this In that case, one represents the highest difference, and zero represents perfect correspondence.

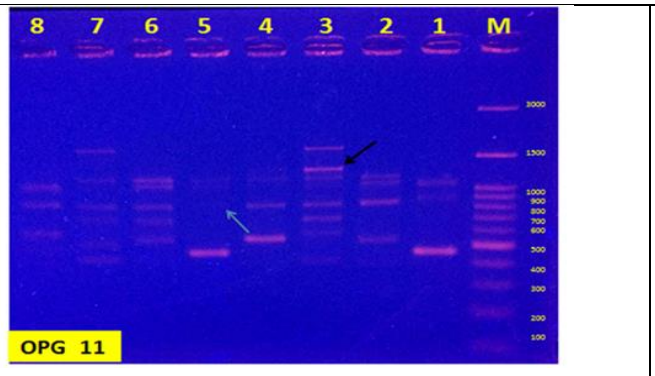
It was shown through the genetic distance values indicated in Table (7) that the genetic distance values ranged between (0.275 - 0.664), where the lowest genetic distance was between the two varieties, Inaa 99 G3 and Al-Nizar G6, where it reached 0.275, and this is considered the highest similarity between the two varieties included in the study. As for the highest genetic distance, it reached 0.664 between the two varieties, Buhouth 22 G5 and Al-Buro G7, and this is considered the lowest genetic similarity between the varieties under search, while a genetic distance values for the rest of the structures ranged between those values.

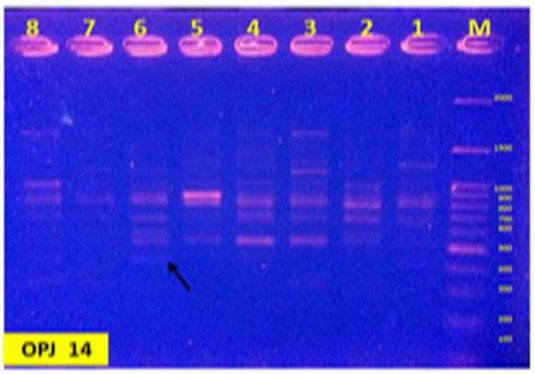
Table 6: Represents the genetic dimension values of the bread wheat varieties

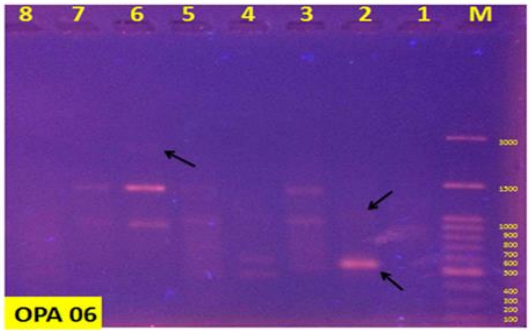
G1 G2 G3 G4 G5 G6 G7 G8
P G1 0.000
P G2 0.432 0.000
P G3 0.576 0.425 0.000
P G4 0.447 0.381 0.339 0.000
P G5 0.489 0.580 0.386 0.401 0.000
P G6 0.613 0.348 0.275 0.353 0.423 0.000
P G7 0.639 0.405 0.530 0.497 0.664 0.535 0.000
G8 0.446 0.329 0.359 0.381 0.377 0.370 0.517 0.000

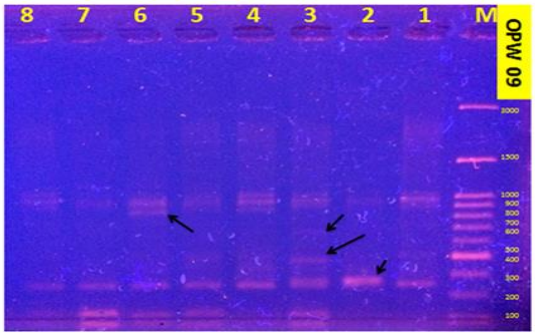
Figure (1) shows the genetic relationship between eight varieties based on the results of RAPD-PCR

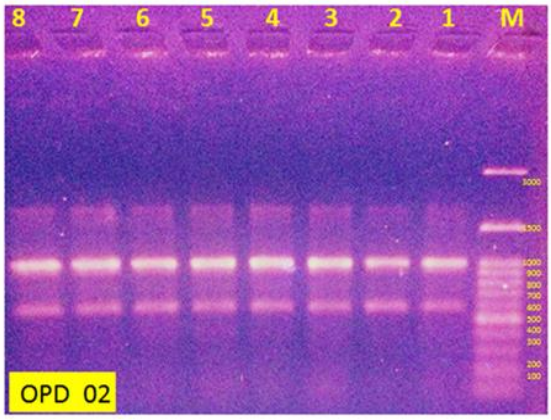


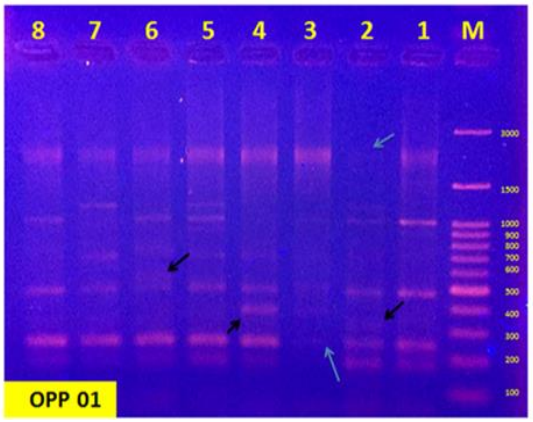












Figures (1-6) represent the products of primer amplification with the DNA of the eight bread wheat varieties, staged on a 1.5% agarose gel with volumetric index M.

DISCUSSION

The importance of genetic variation gained from the resulting packages of RAPD-PCR is of particular importance to genetic fingerprinting manuscripts because it reduces the effort and resources required to achieve its goals with the least possible interaction. (Yadav *et al*., 2019). This aligns with a study (Tahir, 2015) in which (18) primers were used, (16) of which produced heterozygous bands and (2) produced heterozygous bands. It produced general bundles for ten genotypes of wheat plants, and this is consistent with most researchers who have used RAPD markers in studying genetic variation (Grewal *et al*., 2007; Vishwanath *et al*., 2010; Fadoul *etal*., 2013 and AL-Tamimi, 2014).

One of the foundations upon which RAPD indices are based is the number of bands that appear in the genome of any sample, which in turn represents the number of sites that the primer finds and binds to. The number of these sites is affected by two main factors: the size of the genome under study and the primer sequences. Accordingly, estimated statistics in this field indicate that short random primers (9-10 bases) can find (2-10) sites on the DNA of the genome of higher organisms such as plants (Kumar *et al.*, 2017; Eid, 2019).

These bands are a distinctive and diagnostic feature of these genotypes. Their presence in only one genotype indicates a mutation at a specific locus that led to the recognition of the primer and the emergence of this unique band. Conversely, the absence of these bands indicates a similar mutation at the primer recognition locus in only one genotype, resulting in the cancellation of that locus. The emergence of these bands, consistent with the findings of many previous researchers, is a key focus for DNA fingerprinting researchers because it reduces the effort and resources required to achieve their goals with fewer interactions (Celka *et al*., 2010; Sharifova *et al*., 2013). This opens up future possibilities not only for identifying distinctive bands for other genetic constructs but also for linking these segments to other field or analytical characteristics to facilitate the work of plant breed (Kumar *et al*., 2017; AL-Tamimi, 2014).

Genetic similarity is a measure of the degree of genetic compatibility between any two variation . Therefore, the results are equal when there's no genetic variation between the genotypes. This is evident when the individuals share the same general genetic makeup and no distinct genetic makeup is present between them.. The use of a small number of primers reduces the significance of such results, but they become more valuable when the number is increased, as in this study (Salehi *et al*., 2018). This is consistent with the use of a number of primers in this study, which had the ability to distinguish the variation between the studied genetic structures through the different bands resulting from this number of bands that scanned the largest possible portion of the broad bean genome plant breeders (Celka *et al.,* 2010: Islam *et al*. 2012: Sharifova *et al.,* 2013).

CONCLUSION

This research concludes that there is genetic variation among the studied varieties, as well as the efficiency the RAPD marker in determining the genetic fingerprint of all varieties, in addition to determining the genetic distance between varieties and determining the degree of genetic relatedness. It concludes that the more primers used, the more efficient the indicator is in determining the genetic fingerprint and determining the genetic distance. What determine the genetic proximity or distance between the studied varieties is the number of shared bands. The more of these bands, the less the genetic distance and vice versa. These shared bands indicate a similarity in the genetic material in that region of the genome of the studied varieties, which may represent a similarity in phenotypic traits, especially the quantity of the crop, or in other traits related to productivity, reproduction, and disease resistance, or a similarity in genetic adaptation to the environmental requirements suitable for growth and production, or many other traits. Or the similarity may be in a region of non-coding regions, i.e., those that do not have gene expression and are known as Non-coding DNA.

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