Study of genetic dimension for local varieties of bread wheat

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Abstract: In this study, nine types of bread wheat (*Triticum aestivem* L.) were used. To analyze it at the molecular level, Random Amplified Polymorphic D.N.A. (R.A.P.D.) markers based on PCR technology were used to study the variations of the cultivars. It is absent in other varieties and can be used as a distinctive genetic fingerprint to preserve plant breeders' rights. Genetic banks can use it to distinguish the mentioned varieties and models, confirming the importance of this technique in molecular characterization and genetic fingerprinting studies. From the results of the molecular aspect, the similarity matrix was implemented based on the coefficients of (Nei & Lie) for the nine studied varieties.

The highest similarity value was between the variety (Taymoz/2 and Baghdad/3), and its value was 0.9167. Most of the traits showed a similarity in the mean between them for all the genetic traits, but the lowest similar value was between the two varieties (Rashid and Baghdad/1). Genetic level, the cultivar seeds were sown under arid conditions in the fields. The experimental unit contained two lines for each category, the length of the line was 2 m, and the distance between the lines was 30 Cm. The characteristics of the number of days of 50% flowering, plant height (Cm), biological yield (gm/plant), grain yield (gm/plant) and weight of 100 grains (gm) were studied. The inputs differed significantly at the 1% probability level for all studied traits. There was high genetic variance for the characteristics of the biological yield (kg/ha) and seed yield (kg/ha), and the weight of one hundred seeds (g). The highest values of genetic variation coefficients for seed yield were superior. The percentage of heritability in its broad sense was high for all the studied traits. The values of the expected genetic improvement were a high percentage of seed yield, vital yield, harvest index, number of total branches per plant, the average for plant height, first pod height (Cm), number of seeds/pod, the weight of 100 seeds, and few numbers of days to flowering at 50%.

Key words: Triticum aestivem, R.A.P.D., Phylogenetic, Molecular markers.

Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most important cereal crops globally and the most cultivated and productive. The area planted with wheat in 2018 reached 214 million hectares, and its productivity was about 34.254 tons/hectare[1]. In Iraq, the area planted with wheat reached about 6331 thousand dunams for the winter season of 2019, and its production capacity for the same year is about 4,343,000 tons[2].

The study of genetic parameters has a preliminary indicator for evaluating the varieties and the extent of variation between them. Several researchers estimated these genetic parameters in the wheat crop were estimated by several researchers where (3) indicated that the values of genetic, phenotypic, and environmental variations were significantly high for plant height and leaf area. The environmental variance values were higher than the genetic variance values. (4) reported that the values of genetic variance were significant for most of the traits except for the traits of several days to flowering, the number of spikes/m², biological yield g/m², and grain yield g/m². Grains/spike and the weight of 1000 grains in bread wheat.

(4) showed that the phenotypic variation coefficient va-

lues were high for grain yield, g/m², medium for the number of spikes/m², biological yield, g/m², and the number of grains/spike, and low for the rest of the traits in bread wheat. As for the genetic variation coefficient values were high for the number of grains/spike and grain yield g/m² and the average for the rest of the traits, and the values of the phenotypic variation coefficient were more significant than the genetics for all traits.

(3) showed that the values of heritability in the broad sense were high for plant height, the number of spikes per plant, The numerical quantity of wheat grains in the spike grain yield and weight of 100 grains, and (5) indicated that the expected genetic improvement values were high for the characteristics of grain weight/spike, plant height, 1000-grain weight, and spike length in coarse wheat.

Since the R.A.P.D. Technique high efficiency has been used Widely in the genetic variation analysis (6) have estimated the genetic variation using 23 random primers with various bread wheat varieties, and they found that gene diversities were 0.8028 and 0.927. Tease high values indicate that the random primers used in the R.A.P.D. can identify the variation between the wheat varieties depending on the

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presence of the prominent bands and their numbers. (7) has reported that the primers he used showed an apparent genetic variation among the refined wheat under study. 8reported the genetic distance for (8) tetraploid wheat varieties using 24 random primers; two of them showed standard bands while 22 primers showed 141 bands, 101 of them were variable. The genetic distance percentage was 72.6% using R.A.P.D. markers.

The study of the genetic dimension is important because it is one of the goals that plant breeders aspire to evaluate varieties for each breeding program, whether (selection, crossbreeding, introduction), and to locate quantitative traits and preserve genetic assets, in addition to that it does not require hypotheses about the nature of the data distribution, and The reflection of the field assessment of varieties with the laboratory through the use of polymorphic random multiplication indicators[9].

This study aims to evaluate the performance of cultivars and their adaptation to environmental conditions by estimating genetic parameters and estimating the degree of genetic divergence between the studied cultivars.

Materials and methods

The study included two phases:

The field study

The study was carried out in the fields of the College of Agriculture and Forestry, University of Mosul, and included the cultivation of seeds of fifteen varieties of bread wheat (*Triticum aestivum* L. sourced from the Plant Breeding Center / Ministry of Science and Technology - Baghdad and Table (1) show the genealogy of the varieties. They were planted on 16/12 /2018, according to the design of complete randomized sectors. RCBD[10], with three replications, where the items were randomly distributed in each repetition and by two lines for each item. The line length was 2 meters, the distance between the lines was 30 cm, and urea fertilizer was added at a rate of 20 kg (nitrogen). per dunum[11], which is equivalent to 43.47 kg of urea, in two batches, the first - when planting and the second before the spikes were expelled. The study was conducted on 10 plants taken randomly, excluding the terminal plants from each line.

The amount of rain falling during the growing season was (812.5 mm). The study was conducted on 10 plants randomly taken from every two lines, and the following characteristics were studied:

1- The number of days from planting until 50% of the spikes left the flag leaf sheath.

2- Plant height (Cm): The height of the plants was measured from the surface of the soil to the top of the ears, without the ridge.

3- The length of the spike (Cm): It was measured from the base of the spike to the top of the spike without the spur estimated.

4- The number of spikes/m².

5- The number of grains of a spike: the average number of grains in each spike were calculated.

6- The vital yield: It represents the weight of the product of two lines (spikes + straw) and converted to kg/ha.

7- Grain yield: This is from the yield of two lines and then converted to kg/hectare.

8- Harvest index (%): It was calculated using the formula: Harvest index % = (grain yield/biological yield) x 100 mentioned by (12).

9- The weight of 1000 grains is estimated in grams. The data were statistically analyzed according to the randomized complete block design (R.C.B.D) using the S.A.S. program (2004); genetic, phenotypic and environmental variances were estimated according to the method explained by (13) according to the randomized complete block design (R.C.B.D) as reported by (10).

$$\sigma^2{}_g = (M.S.t - M.s.e)/r \qquad \sigma^2{}_e = M.S.e \qquad \sigma^2{}_p = \sigma^2{}_g + \sigma^2{}_e$$

As for the standard error of the phenotypic variance, it was estimated according to what was mentioned by (14) according to the equation:

$$SE(\sigma^2 G) = \sqrt{\frac{1}{r^2}\left[\frac{2(msg)^2}{k+2} + \frac{2(mse)^2}{k+2}\right]} \quad SE(\sigma^2 E) = \sqrt{\frac{2(mse)^2}{k+2}} \quad SE(\sigma^2 p) = \sqrt{\frac{2(\sigma^2 p)^2}{N}}$$

Note that k = degrees of freedom for each source (genotypes or experimental error), r = the number of replicates, where N = the sum of the degrees of freedom for genotypes and experimental error, and the values of the coefficients of phenotypic variation (PCV) and genetic variation (G.C.V.) were calculated according to the method explained by (15), and based on the ranges used by (16) it is less than 10% low, 10-30% medium, and more than 30% high.

$$PhenotypicCoefficientofVariation(P.C.V.) = \frac{\sqrt{\sigma^2{}_P}}{\overline{X}} \times 100$$

	Genotype	Source
1	Sally	Plant Breeding Center, Ministry of Science and Technology, Baghdad
2	Taymoz/2	Plant Breeding Center, Ministry of Science and Technology, Baghdad
3	Rashid	Plant Breeding Center, Ministry of Science and Technology, Baghdad
4	Abu Ghraib	Plant Breeding Center, Ministry of Science and Technology, Baghdad
5	Sham	Plant Breeding Center, Ministry of Science and Technology, Baghdad
6	Baghdad/1	Plant Breeding Center, Ministry of Science and Technology, Baghdad
7	Babylon/113	Plant Breeding Center, Ministry of Science and Technology, Baghdad
8	Baghdad/3	Plant Breeding Center, Ministry of Science and Technology, Baghdad
9	Adana	Plant Breeding Center, Ministry of Science and Technology, Baghdad

Table 1. Genealogy of genotypes and their numbers used in the study

And finding the values of inheritance in the broad sense in the way they explained[17]. The ranges explained by (18) were adopted. The values of heritability in the broad sense are less than 40%, low, 40-60% is medium, and 60% or more are high.

$$Genotypic\,Coefficient\,of\,Variation\,(G.C.V.) = \frac{\sqrt{\sigma^2_g}}{\bar{X}} \times 100$$

$$H^2 = \frac{\sigma^2_G}{\sigma^2_P}$$

The expected genetic improvement when 5% of the plants were selected was estimated[19]. The ranges suggested by (16) for the expected genetic improvement were adopted as a percentage of the average: less than 10% is low, 10-30% is medium, and more than 30% is high.

The Laboratory Study

Although the R.A.P.D. The technique is a relatively old and simple molecular method, but it is still widely used due to its ease of application and economics compared with other high accuracy techniques.

DNA Extraction

Genomic D.N.A. was extracted from plant tissue according to the protocol of Wizard Genomic D.N.A. Purification Kit (Promega, U.S.A.).

Quantitation of D.N.A.

To detect the extracted D.N.A., a fluorescent indicator was used to detect the goodness of samples for downstream applications. For 1 µl of D.N.A., 199 µl of diluted Quanty Flour Dye was mixed. After 5min incubation at room temperature, D.N.A. concentration values were measured.

PCR Amplification

Thermal Cycling Protocol

Gene: ITS

No. of Reaction	48	rxn	Annealing temperature of primers	55
Reaction Volume /run	10	µl	Length of PCR product (bp)	729bp
Safety Margin	5	%	No. of PCR Cycles	30

Table 2. Thermal Cycling Protocol.

Primers

Primer Name	Seq.	Tm	Product Size
ITS1	TCCGTAGGTGAACCTGCGG	55	729bp
ITS4	TCCTCCGCTTATTGATATGC		

Table 3. Primers.

RAPD Technique

The R.A.P.D. The technique was applied using nine random primers in the sequences shown in table (4) to study the discrepancy between 9 varieties of wheat entering Iraq for the first time (Table 1).

T	Primer	Sequence
1	R1	GGTTCGGGAA
2	R2	GAAACGGGTG
3	R3	CACACTCCAG
4	R4	CAAACGTCGG
5	R5	GCATATCCGG
6	R6	CTGACCAGCC
7	R7	ACCAGGTTGG
8	R8	GGACCCCGCC
9	R9	CTTTCGTGCT

Table 4. Names and sequences of the random primers used in the experiment.

Agarose Gel Electrophoresis

After PCR amplification, agarose gel electrophoresis was adopted to confirm the presence of amplification at 80 volts for an hour and 40 minutes. The results of the R.A.P.D. Random multiplication was examined by imaging the electrophoresis patterns for each primer and identifying the bands, where the presence of the bands is represented by the number (1) and the absence of the band by the number (0). Cluster analysis was also conducted using the Euclidean program, and the scale through the genetic distance was estimated according to the R.A.P.D. Indicators and based on the similarity scale (Jaccard Index), after calculating the similarity ratios according to the previous laws, the other stage begins to calculate the genetic kinship and the Phylogenic evolution tree was drawn for a class of similar individuals next to each other in one cluster. If the genetic material is identical between two classes, this indicates that the genetic dimension must be equal to zero, and the similarity ratio is equal to 1 (100%)[20].

Results and discussion

It is noted from Table (6) that the averages of the cultivars' squares differed significantly at the 1% probability level for all the studied traits except for the trait of the number of days to flowering at 50%, which did not reach the level of significance. Such differences between varieties in the characteristics give the appropriate opportunity for plant breeders to benefit from them in future breeding programs.

Table (7) indicates the averages of the cultivars and for nine studied traits, during which it becomes clear to us that there are differences in these cultivars and for all traits, where the cultivar Adana and in the desired direction outperformed the rest of the cultivars in the short period of days for flowering at 50% as it reached (94 days). As for the plant height characteristic, the cultivar Sally showed its superiority in the desired direction over the rest of the cultivars in length and reached (101.733 cm). The characteristic of the area of the flag leaf showed the highest value in the variety Babylon/113 in the desired direction, as it reached (42.079 cm^2). The cultivar Sally showed its superiority over the rest of the cultivars and in the desired direction, as it reached a value of (5.800) for the characteristic of the number of spikes/plant thus it is possible to benefit from the cultivar Sally by including it in the crossbreeding programs. It is noted that the highest value of the average spike length was in the cultivar Babylon/113 in the desired direction, and it reached (12.367 cm). As for the characteristic of the number of grains/spike, the Rashid variety excelled in the desired direction and reached (68,333) over all other varieties, and from this, it is possible to benefit from the variety Rashid in improving the grain yield of gm/plant by increasing the number of grains/spike.

The genetic structure of Sally gave the highest value in the trait of biological yield, g/plant, and in the desired direction, which amounted to (23.415 g). The results of the average values of the grain yield, g/plant, indicate the superiority of the cultivar Sally over the rest of the cultivars and in the desired direction, as it reached (7.805 g). Therefore, it is possible to select the variety Sally and replanted in subsequent studies and in multiple locations to ensure its superiority and stability of its characteristics, to benefit from it either directly as a new variety or by entering it into crossbreeding programs with other varieties. As for the 1000-grain weight

Steps	Temperature °C	Time	Cycle no.
Initial Denaturation	95	5 min.	1
Denaturation	95	45 sec.	35
Annealing	35	1 min.	
Extension	72	1 min.	
Final extension	72	7 min.	1
Hold	4	5 min	1

Table 5. PCR Program.

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traits Sources	Degrees of freedom	Number of flowering days at 50%	Plant height (cm)	Flag leaf area (cm²)	Number of spikes/plant	spike length (cm)	Number of grains/spike	biological yield (gm)/plant	grain yield (gm)/plant	weight of 1000 grains (gram)
Repeats	2	0.111	506.139	116.432	14.801	9.731	53.444	66.830	7.425	42.884
varieties	8	1.083 n.s.	424.495 **	201.084 **	4.469 **	15.758 **	57.666 **	47.664 **	5.296 **	58.512 **
error	16	0.736	63.866	46.606	0.611	1.398	11.361	8.089	0.899	11.048

Table 6. Analysis of variance for the studied traits in bread wheat. **** Significance at 0.01

trait, Rashid showed a high superiority over the rest of the cultivars in the desired direction and reached (60.564 g); and thus, Rashid could be entered into the crossbreeding program with other cultivars to transfer the genes of the high yield, as the 1000-grain weight trait is one of the main yield components.

Table (8) shows that the values of phenotypic, genetic and environmental variances were significant from zero for all studied traits except for the number of days to flowering at 50%. Environmental variations were significant for most traits except for the traits of a number of days to flowering at 50%, several spikes/plant, spike length, and the grain yield, and concerning the phenotypic variations, it was significant for all traits except for the number of days to flowering at 50%, the number of spikes/plant and the grain yield and this is in line with what was mentioned by (21),(22) and (4) and (23) As for the coefficients of phenotypic and genetic variation, they were medium and low for most of the traits, and these results are in line with what was reached by4. As for the values of heritability in the broad sense, they were high for the characteristics of plant height, the number of spikes/plant, spike length, biological yield and grain yield, medium and low for the rest of the traits is consistent with (3). The expected genetic improvement values were high for the characteristics of the leaf area/cm², the number of spikes/plant, spike length; biological yield, grain yield, medium and low for the rest of the traits, and these results are in line with what[4,5].

PCR expansion

Figure. 1 ITS region amplification fractionated on 1% agar gel electrophoresis stained with Eth.Br. M: 100bp D.N.A. marker.

Polymorphism resulting from the use of R.A.P.D. technique

Eight primers were highly efficient in detecting the divergences between the varieties, according to Table (9).

Depending on the primer used, the results showed a clear difference in the number of alleles, in addition to the presence of primers that did not show any bands, and the reason may be due to the absence of a complementary linkage region for the sequence of bases for this primer on a complementary site within the D.N.A. sequences of the genome of the studied species. Several studies showed the presence of differences in results for primers[24,25]. The rest of the primers had varying abilities to detect the magnitude of genetic variation among the studied genotypes, while others gave a less number of bands. We also note that the nine primers gave 30 allelic loci, including 9 major alleles for their appearance in all genotypes, while the different alleles are of the characteristic alleles of the genotypes, and 21 alleles are relied upon to determine the genetic link between the genotypes.

Primer R1

This primer was used in genotypic D.N.A. replication, and 4 different allelic binding sites were obtained, and the percentage of them was 100%. This primer showed an apparent variation in bands position and a molecular weight ranging between 1000-1100bp.

From Figureure (1), it is clear that cultivars 6 and 4 do not have any linkage site with this primer, and the reason is due to the absence of a complementary linkage region for the base sequence of this primer on a complementary site within the genome D.N.A. sequences of the studied species.

Primer R2

The primer was able to identify 3 sites of its complementary sequences in the D.N.A. of the genome; all of the first structures with a size of 35 bp and the second 1100 bp are present in all varieties. The third link site has complementary sites in the first and second variants, with a size of 1000 Pb. This primer showed a 33.33% variation.

Primer R3

The number was 3, whose sizes ranged from 390bp to 800bp. Cultivar 6 was distinguished by the absence of bands and was considered a distinctive feature of this cultivar, and the variance ratio was 100%.

Primer R4

This primer has many 32 packets that resulted from 4 alleles, all bands are different, so the percentage of divergent bundles becomes 100%. The primer was able to

Genotypes	Number of flowering days at 50%	Plant height (cm)	Flag leaf area (cm²)	Number of spikes/plant	spike length (cm)	Number of grains per spike	grain yield (gm)/plant	biological yield (gm)/plant	weight of 1000 grains (gram)
Sally	95.000	101.733	24.497	5.800	8.567	56.667	23.415	7.805	48.089
Taymoz/2	95.333	61.200	23.034	2.533	9.267	62.667	10.662	3.554	54.652
Rashid	95.000	80.933	41.097	3.200	15.900	68.333	15.836	5.279	60.564
Abu Ghraib	94.333	76.400	31.163	5.133	9.100	63.333	18.743	6.248	54.623
Sham	94.333	77.467	27.845	4.467	10.167	57.333	17.471	5.824	48.786
Baghdad/1	95.000	72.200	37.180	3.267	11.267	59.000	21.789	7.263	51.652
Babylon/113	96.000	83.067	42.079	4.933	12.367	67.333	17.789	5.930	58.775
Baghdad/3	95.000	71.733	21.236	2.467	9.733	60.333	12.811	4.270	52.280
Adana	94.000	64.000	22.645	3.133	9.467	57.000	18.295	6.098	48.986
Average	94.889	76.526	30.086	3.881	10.648	61.333	17.423	5.808	53.156
L.S.D (0.05)	1.485	13.833	11.817	1.353	2.047	5.834	4.923	1.641	5.753
L.S.D (0.01)	2.046	19.060	16.282	1.864	2.820	8.039	6.783	2.261	7.927

Table 7. Average varieties of bread wheat for the studied traits.

Traits genetic parameters	Number of flowering days at 50%	Plant height (cm)	Flag leaf area (cm²)	Number of spikes/plant	spike length (cm)	Number of grains per spike	biological yield (gm)/plant	grain yield (gm)/plant	weight of 1000 grains (gram)
genetic variance	0.116 ± 0.696	120.210 ± 12.825	51.493 ± 5.231	1.286 ± 0.125	4.787 ± 0.388	15.435 ± 1.828	13.192 ± 2.163	1.466 ± 0.240	15.821 ± 1.880
Environmental variance	0.736 ± 1.461	63.866 ± 31.964	46.606 ± 23.346	0.611 ± 1.447	1.398 ± 1.578	11.361 ± 5.854	8.089 ± 4.285	0.899 ± 1.484	11.048 ± 5.702
Phenotypic variance	0.852 ± 4.023	184.076 ± 92.125	98.099 ± 49.212	1.897 ± 4.111	6.185 ± 5.056	26.796 ± 13.982	21.281 ± 11.367	2.365 ± 4.171	26.869 ± 14.017
genetic variation coefficient	0.349	12.533	13.082	5.756	6.705	5.017	8.701	5.024	5.456
phenotypic variation coefficient	0.947	15.509	18.057	6.991	7.621	6.610	11.052	6.381	7.110

Table 8. Genetic parameters of the studied traits in bread wheat.

identify the complementary sequences in the D.N.A. of the genome for the genotypes, and it showed an apparent variation in the molecular weight ranging between 850-1700 bp; from Figureure (4) we note that the primer did not find its complementary series at genotype 3, so no bundle appeared; thus it can be considered as an imprint of this genotype in this prim.

R5

This primer distinguished variant 1 with a band size of 1700 bp. It also able to identify the complementary sequences in the D.N.A. of the genome, as it showed a clear variation in the molecular weight, ranging between 600-300 bp.

R6

This primer showed two complementary genomic loci, the first was associated with a size of 800 bp, and the second site distinguished a locus with a size of 1500 pb. As for variants 2 and 6 respectively, it was distinguished by the absence of any complementary linkage to the primer, and the divergence ratio was 100%.

R7

This primer was distinguished by the presence of 4 identical bans for all types, the similarity rate was 0%, and their sizes ranged from 900 to 1700bp.

R8

This primer produced 5 different bands, ranging from 400 to 1700bp, with a variance of 100%.

R9

This primer produced 3 bands ranging from 400 to 1200pb. Variant 8 was distinguished by the absence of a

Primer	Binding site	Polymorphic	Polymorphism (%)	monomorphic	Bands size
R1	4	4	100	0	900-1000
R2	3	1	33.33	2	35-1000
R3	3	3	100	0	800-390
R4	4	4	100	0	850-1300
R5	1	1	100	0	1300-600
R6	2	2	100	0	800-1150
R7	4	0	0	4	900-1300
R8	5	5	100	0	300-1300
R9	4	1	66.6	3	400-1200
	30	21			
	3.33	2.33			

Table 9. Genetic variances for the primers used.

band with a size of 400pb in all varieties, and the variance ratio was 66%. This is considered a distinctive feature of variant 8 because the initiator did not find a complementary site for it.

The relative relationship between the wheat cultivars under study based on R.A.P.D. data

According to the previous results, the R.A.P.D. The technique was able to find unique and distinct bands capable of distinguishing between the studied wheat varieties; that is, these bundles were found in a particular variety and absent in other varieties. Genetic banks can use it to distinguish the mentioned cultivars. This confirms the importance of this technique in studies of molecular characterization and genetic fingerprinting.

A genetic relation tree was made for the nine taxa, where U.P.G.M.A. similarity values were estimated using the studied R.A.P.D. method based on data analysis.

The similarity between the studied wheat cultivars was Estimated based on the results shown by the primers, where the results that appeared in the gel electrophoresis were transferred to the characterization tables by placing one in the presence of a band and zero in its absence after that the symmetry matrices are formed based on the coefficients Table (3) results of the similarity matrix between the studied varieties. The highest similarity was between the variety (2 and 8), and its value was 0.9167; most of the traits showed closeness in the arithmetic means between them for all genetic traits, while the lowest similarity value was between the two variants (3 and 6), where the result of reading the arithmetic mean was significantly different in most of the phenotypic traits between these two variants, these results between varieties using R.A.P.D. Indices were associated with phenotypic traits and were consistent with what was found before[8], where the results showed that the lowest genetic distance was between two cultivars that have specific significant characteristics in common.

Cluster analysis of genotypes of bread wheat based on R.A.P.D. Markers

Cluster analysis shows the evolutionary relationship of a group of living organisms that arose from a common lineage containing the largest possible number of genotypes in plant breeding and development programs,. The accessions may be collected in one group according to their geographical location or origin and lineage Figureure (11).

The nine bread wheat varieties cluster tree showed two main groups; the first leading group was divided into two secondary groups, and the first secondary group was divided into two branches. The genetic material of these genotypes may be similar according to the number of D.N.A. segments to the gene of these complementary structures of the primer sequences used, while the second secondary group included variants 4 and 6, and the reason for this relationship is due to sharing most of the genetic traits and their possession of a number of similar D.N.A. pieces to show the extent related to each other, while the second main group included only variants 3 and 9, due to their uniqueness of their behavior with most of the primers used in the study.
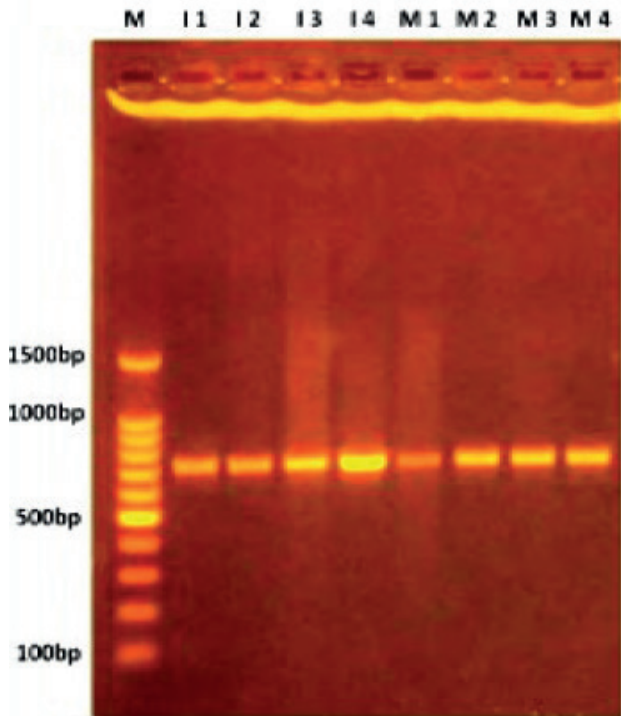
Conclusions

Among the results obtained from both aspects, the molecular study proved its effectiveness of and usefulness for plant breeders and the as the building block for the success of any plant breeding program, and the shortening of time in determining the appropriate varieties to conduct crosses and improving the characteristics of the yield.
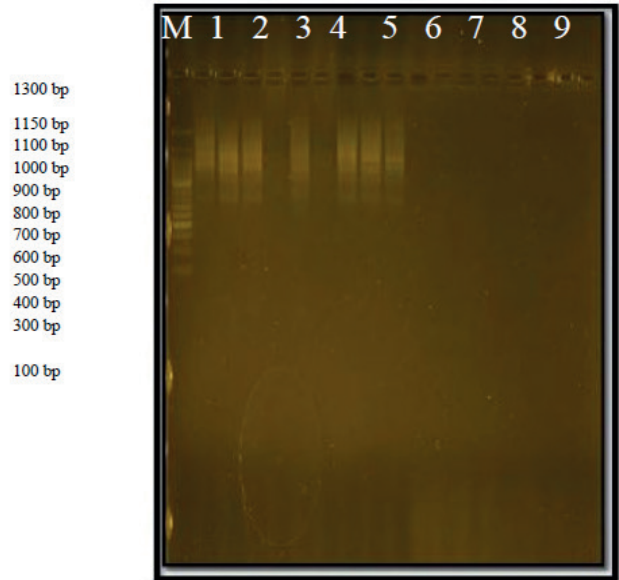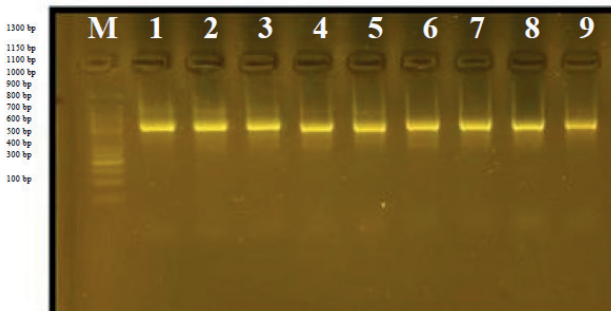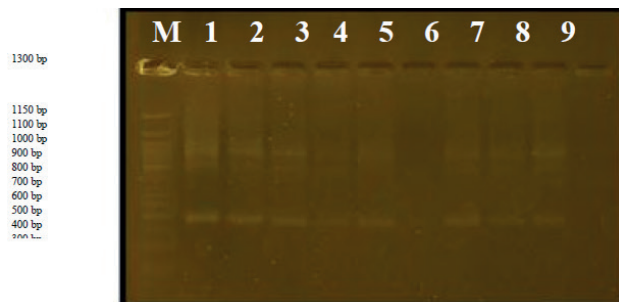
Figure 1. ITS region amplification fractionated on 1% agar gel electrophoresis stained with Eth.Br. M: 100bp D.N.A. marker.
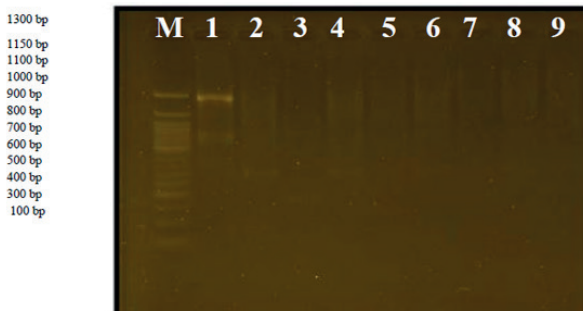

Figure 2. The product of the random amplification reaction of D.N.A. using the R1 primer.
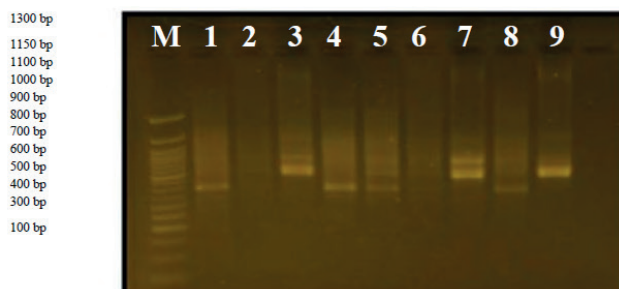

Figure 3. The product of the random amplification reaction of D.N.A. using the R2 primer.

Figure 4. The product of the random amplification reaction of D.N.A. using the R3 primer.

Figure 5. The product of the D.N.A. random amplification reaction using the primer R5.

Figure 6. The product of the DNA random amplification reaction using the primer R6.
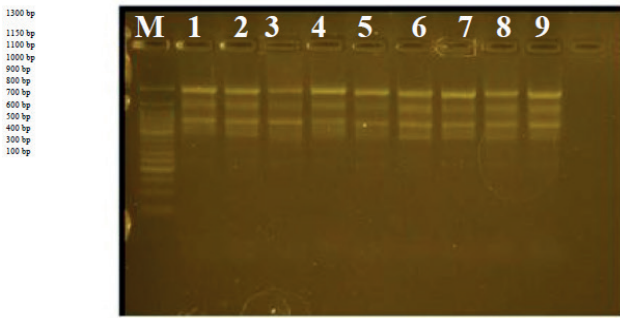
Acknowledgments

Conflicts of Interest

There is no conflict.

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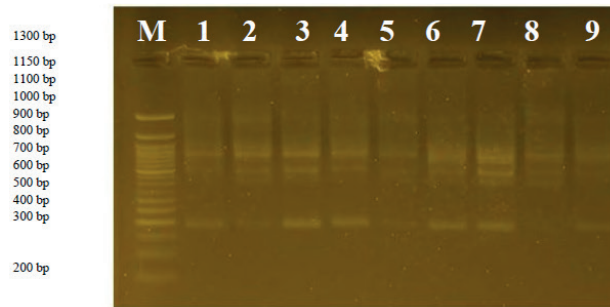
Figure 7. The product of the D.N.A. random amplification reaction using the initiator R7.
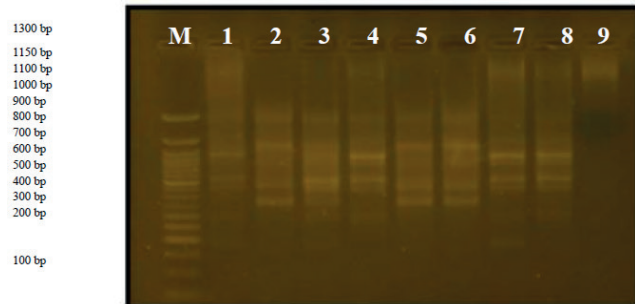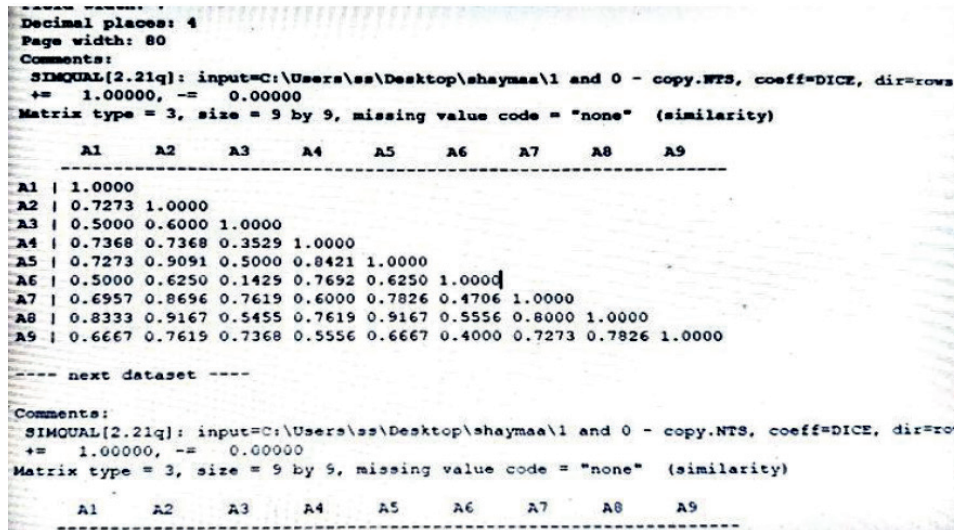

**Figure 8.** The product of the D.N.A. random amplification reaction using the initiator R8.

Figure 9. The product of the D.N.A. random amplification reaction using the initiator R9.


```
Decimal places: 4
Page width: 80
Comments:
 SIMQUAL[2.21q]: input=C:\Users\ss\Desktop\shaymaa\1 and 0 - copy.NTS, coeff=DICE, dir=rows
 +=   1.00000,  -=   0.00000
Matrix type = 3, size = 9 by 9, missing value code = "none"  (similarity)

        A1      A2      A3      A4      A5      A6      A7      A8      A9
     -----------------------------------------------------------------------
A1 | 1.0000
A2 | 0.7273 1.0000
A3 | 0.5000 0.6000 1.0000
A4 | 0.7368 0.7368 0.3529 1.0000
A5 | 0.7273 0.9091 0.5000 0.8421 1.0000
A6 | 0.5000 0.6250 0.1425 0.7692 0.6250 1.0000
A7 | 0.6957 0.8696 0.7619 0.6000 0.7826 0.4706 1.0000
A8 | 0.8333 0.9167 0.5455 0.7619 0.9167 0.5556 0.8000 1.0000
A9 | 0.6667 0.7619 0.7368 0.5556 0.6667 0.4000 0.7273 0.7826 1.0000

---- next dataset ----

Comments:
 SIMQUAL[2.21q]: input=C:\Users\ss\Desktop\shaymaa\1 and 0 - copy.NTS, coeff=DICE, dir=row
 +=   1.00000,  -=   0.00000
Matrix type = 3, size = 9 by 9, missing value code = "none"  (similarity)

        A1      A2      A3      A4      A5      A6      A7      A8      A9
     -----------------------------------------------------------------------
```

Figure 10. The kinship relationship between the studied wheat cultivars based on R.A.P.D. data.
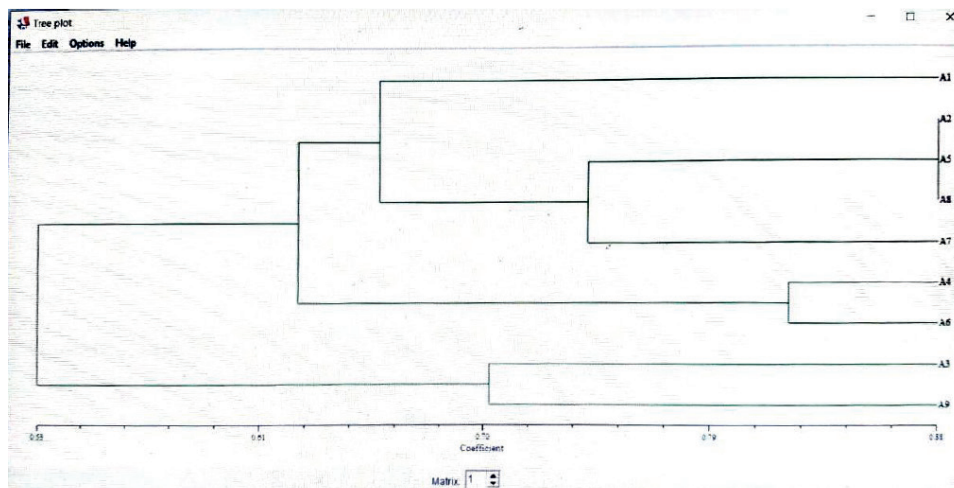
Figure 11. Neighbor-joining tree for the studied varieties using unequal pairs groups (U.P.G.M.A.).

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