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## QUALITATIVE CHARACTERISTICS AND GENOMIC ANALYSIS OF WHEAT GENOTYPES IN IRAQ

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### SUMMARY

The breeding material comprising 17 Iraqi wheat cultivars belongs to three different types, i.e., a) salinity-tolerant cultivars (Dajla, Furat, 1H, 2H, 2N, 3H, 3N, and 7H), b) drought-tolerant cultivars (Sham-6 and Orok), and c) local cultivars (Iraq, Iba99, Iba95, Abu Ghraib-3, Adnanin, Tamoze, and Alrashid) underwent qualitative characteristics and genomic analysis studies in 2021-2022, at the Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq. Measuring the percentage of protein, wet and dry gluten, and molecular fingerprinting used the randomly amplified polymorphic of DNA (RAPD) technique with six primers, with traits estimation using a dendrogram. The highest percentage of protein (24.5%), wet (52.7%), and dry gluten (27.3%) emerged from the wheat genotype Dajla. However, the recorded lowest percentages of wet (32.52%) and dry gluten (7.62%) appeared in wheat genotype Iba99. The cultivars Aadnania, Abu Ghraib-3, and Tamoze gave the lowest protein content of 9.45, 10.34, and 10.54, respectively. The cluster analysis divided 17 wheat genotypes into two large cluster groups. Amplification of all 365 loci used six primers. Fragments' size ranged from 100 bp to 2000 kb. The highest number of bands (73) was amplified with primer Pr-5, while the lowest number (48) was with primer Pr-1.

**Keywords:** Wheat (*Triticum aestivum* L.), salinity- and drought-tolerant genotypes, qualitative characteristics, RAPD primers, genomic analysis, cluster analysis, and phylogenetic tree

**Key findings:** Out of 17 wheat genotypes, cultivar Dajla showed the best performance by having an outstanding percentage of protein, wet, and dry gluten. The cluster analysis divided 17 wheat genotypes into two large clusters. The most frequent bands were amplified with primer Pr-5, while the lowest was primer Pr-1.

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## INTRODUCTION

Wheat (*Triticum aestivum* L.), considered the predominant staple food crop worldwide, is a leading wheat species, with thousands of cultivars globally. Wheat is the most constant food for human beings in many countries, being a chief source of carbohydrates, minerals (iron, calcium, and phosphorus), essential for strengthening the bones, vitamins A, B, and E, and proteins that support the immune system. It is also high in fibers, strengthening the digestive system. Moreover, wheat is an economically important crop that provides the raw material to various industries and trades. A most cultivated crop, wheat occupies the largest region compared with other cereal crops (FAO, 2015; Al-Falluji, 2018).

According to the International Wheat Production Statistics (IWPS), Iraq has the 22nd position in wheat production, with 6.2 million t produced in 2020 (FAO, 2021). However, the yield has fallen to 4.2 million t per year due to various factors the country faced, most notably drought. According to various agricultural criteria and impacts of many factors, wheat classification has different types (AL-Fatlawi *et al.*, 2022; Babar *et al.*, 2022). Under varied environmental conditions, the instability of the quantitative traits makes it difficult to manage the various polygenic characteristics in wheat (Talaha *et al.*, 2016; Hamza *et al.*, 2020; Utebayev *et al.*, 2022).

For evaluating new and existing genotypes, it is necessary to estimate the influencing factors and determine the extent of their impact on the genetic characteristics and quality of the crop, taking into account the different environments and comparing their performance to the local standard cultivars (Al-Tamimi and Al-Janabi, 2019; Mueen and Jabbar, 2019). Wheat cultivars must have high-yielding potential, as well as, other economically crucial traits in terms of tolerance to drought, salinity, and other abiotic factors, and be acceptable to the farming community (Hamza *et al.*, 2020). Therefore, estimating

genetic and environmental interferences and recognizing the stability of the newly developed genotypes and structures are essential for consideration. In recent years, favorable breeding of wheat cultivars with desirable morphological and yield-related traits has become a new target (Khoshgoftarmanesh *et al.*, 2012). Min *et al.* (2020) findings revealed that the qualitative characteristics of wheat grain differ according to the genetic composition of the variety, where most of the selections that are similar in specific features of wheat are of close genetic divergence.

Molecular biology leads to the evolution of DNA markers, which leads to efficient use to identify several characteristics in genotypes under different environmental conditions. Relatedly, the random amplified polymorphic DNA (RAPD) assay is the most used and easy PCR-based technique for producing molecular markers. The RAPD technique is remarkable for detecting rapid markers associated with valuable genetic variations among wheat genotypes (Mueen and Jabbar, 2019). RAPD analysis may help identify the genetic polymorphism and also detect unique specific locus-specific markers. Therefore, the current study aimed to carry out the cluster analysis in wheat genotypes and estimation of qualitative nutritional properties and their comparison in three different groups of Iraqi wheat cultivars.

## MATERIALS AND METHODS

The study proceeded in 2021-2022 at the Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq. The experimental material used in the research comprised three different types of wheat cultivars, i.e., a) salinity-tolerant cultivars (Dajla, Faurat, 1H, 2H, 2N, 3H, 3N, 7H) (Majeed, 2018); b) drought-tolerant cultivars, i.e., Sham-6 and Orok, and c) local cultivars (Iraq, Iba99, Iba95, Abu Ghraib-3, Aadnania, Tamoze, and Alrahid) (Qadir, 2018), collected from the Agricultural Research Department, Abu Ghraib, Iraq.

### The qualitative characteristics

The qualitative biochemical and quality traits of the three different groups of wheat cultivars gained analysis. The protein content estimation used the Kjeldahl standard AACC (1998), using the constant ( $N \times 5.7$ ). The wet and dry gluten estimates also followed the standard method AACC (1998), using the Glutamic System and dried Glutork 2020.

### Isolation of genomic DNA

Obtaining the DNA from the seeds of wheat cultivars belonging to three different groups used the genomic DNA kit (CATB, Iraq). The purity (1.76 to 2 ng/ $\mu$ l) of extracted DNA measurement used the nano-drop with a wavelength of 230– 280, with the quality of DNA measured using agarose gel to prepare electrophoresis system and red safe stain used for nucleic acid (intron) followed by the UV light to visualize the DNA.

### RAPD assay

This study employed six RAPD Oligonucleotide primers designed by Al-Juboori *et al.* (2018) and synthesized in Bioneer, Korea (Table 1). Dissolving the lyophilized primers in the free deionized distal water obtained a final 10 pmol concentration, with the stock stored at -20 °C. Further, dissolving 10  $\mu$ l from the stock in 90  $\mu$ l of the free ddH<sub>2</sub>O to obtain a total volume of 100  $\mu$ l prepared 10 pmol/ml as dissolved to achieve the final concentration.

### PCR amplification and electrophoresis

PCR work progressed in 20 $\mu$ l total volume consisting of five microliter (PCR Pre-mix/Bioneer) of master mix, two microliter primer (10 pmol/ml), two microliters of DNA templates (100 ng), and 11 microliters deionized water. The operation continued in a thermocycler running the program: one cycle of 94 °C for five min, 40 cycles of 94 °C for one min, 41 °C for one min, 72 °C for two min, and finally one cycle of 72 °C for 10 min. The melting point of each primer determined the annealing temperature.

After the PCR finished, the product separation by electrophoresis used agarose gel prepared by dissolving 1.2 g from agarose powder in 100 ml TBE solution RAPD banding primer used to detect PCR products on 1.2% concentration of agarose gel stained with red safe stain, running for one and half hours in five volts to each cm, then visualized under UV light.

### Data on RAPD result

The band present on the gel received a "1" label, with the band absent labeled as "0." The recorded data for all the genotypes depended on the amplification products and primers and after that, processed with the program SPSS V20. The measurement among the wheat accessions was dependent on the clustering system by UPGMA following the algorithm proposed in past studies (Lance and Williams, 1967).

**Table 1.** The primers with sequences used in the study.

No.	Primers name	Sequences ( '5 - '3)
1	Pr-1	GAGTCAGCAG
2	Pr-2	GTGACGTAGG
3	Pr-3	GGTCCCTGAC
4	Pr-4	CCTGGGCTTC
5	Pr-5	GGGTAACGCC
6	Pr-6	GGACCCAACC

### Genetic distance

The coefficient of genetic distance (genetic distance) and the coefficient of similarity of the studied species estimates used the Neis coefficient. Then, conducting a cluster analysis for it and plotting the genetic distance between the samples and inputs employed the Ni UPGMA method.

$$\text{Genetic Distance (GD)} = 1 - \left( \frac{2 \times NXY}{NY + NX} \right)$$

Where:

GD is an abbreviation for genetics dimension; Nxy abbreviation means the number of beams common between the x and y samples; the Nx symbol refers to the number of total beams in sample x, and Ny is the number of beams in sample y. Depending on the Sneath UPGMA method, drawing the cluster analysis scheme used the Taxonomy (NTSYS-pc) system program to obtain the phylogenetic tree and relations or genetic distance, and thus obtaining the analysis results scheme, which shows the close and distant genetic totals for all studied samples.

### Statistical analysis

Data analysis utilized SPSS statistical software, adopting the *P* value for significant differences.

## RESULTS AND DISCUSSION

Wheat is a valuable food product and consumption crop, based on the increasing population, where countries require high-yielding cultivars to meet nutrient deficiencies (FAO, 2015). It can only be possible through genotypic richness programs of successful breeding (Olgun *et al.*, 2015).

Analysis of variance revealed significant ( $P < 0.001$ ) differences among the wheat genotypes for the quality traits. The percentage of protein among the 17 wheat

genotypes belongs to three different types, ranging from 9.45% (Aadnania) to 24.5% (Dajla) genotypes. A past study also revealed high to low content of proteins in the biochemical analysis of several wheat cultivars (Alhendi *et al.*, 2022).

The highest percentage of wet (52.7%) and dry gluten (27.3%) emerged in wheat genotype Dajla, whereas the lowest wet (32.52%) and dry gluten (7.62%) percentages appeared in genotype Iba99 (Table 2). However, most wheat genotypes have values above 40%, with an average value range of  $42.48\% \pm 0.894\%$ . The wide range of wet and dry gluten may indicate the quality of Iraqi wheat. The wide range of such quality traits might be helpful in terms of determining the correlation with other biochemical and rheological properties. Increasing gluten index indicated the strength of the dough and the similarities with a mean index of dry and wet gluten shown by wheat genotypes (Alhendi *et al.*, 2022).

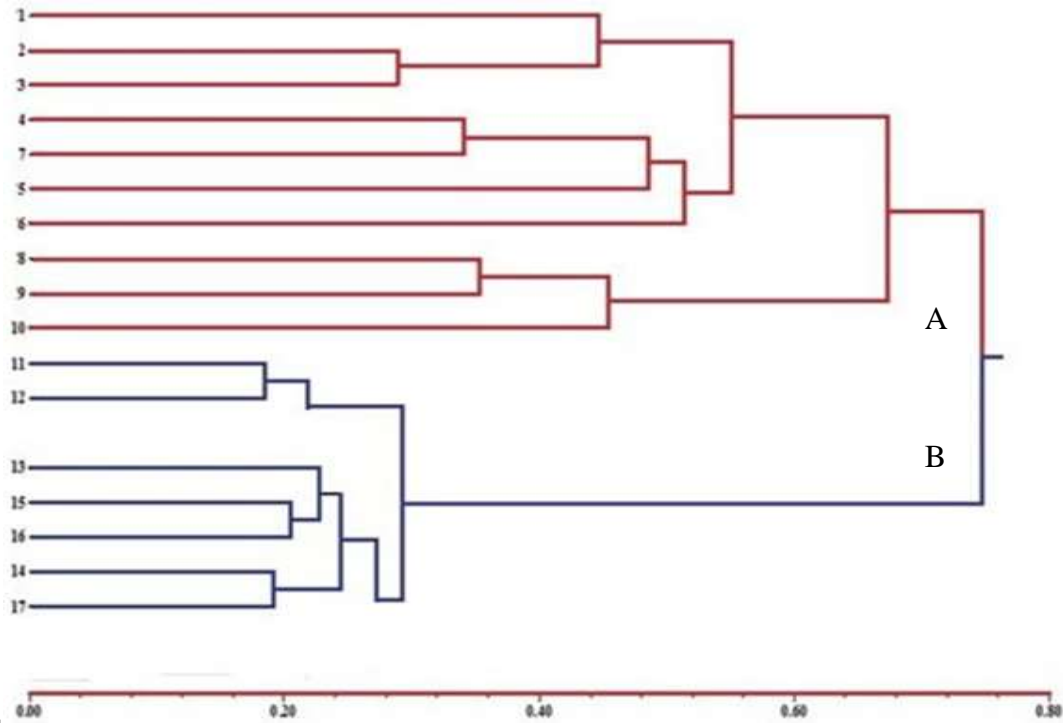
According to the genetic analysis, the extreme genetic space occurred between the landrace Iba99 and 2h (0.99901), followed by Abu Ghraib-3 and 7h (0.92238), and Alrashid and Dajla (0.91697) (Table 3). Conversely, the short genetic space resulted between the genotype Iba99 and Iraq (0.18312), as well as, Al-Rashid and Abu Ghraib-3 (0.19164), and Iba95 and Iba99 (0.21079). The genetic distance refers to the genetic diversity among Iraqi bread wheat genotypes (Al-Juboori *et al.*, 2018). Past studies also revealed similar findings in detecting genetic diversity in bread wheat cultivars through RAPD and ISSR analyses among the different accessions (Olgun *et al.*, 2015) and the molecular database in Iraqi bread wheat cultivars (Mueen and Jabbar, 2019). From Table (2), we note the superiority of the genotypes (m, x) in the specific characteristics of wheat compared with the genotypes (n, t). It is due to the genetic closeness between the genotypes within group A and the genetic divergence between groups A and B (Figure 1).

**Table 2.** Mean performance of the Iraqi wheat genotypes for qualitative traits.

No.	Genotypes	Proteins (%)	Wet Gluten (%)	Dried Gluten (%)
1	Dajla	24.54	52.7	27.3
2	Furat	23.90	50.9	26
3	1h	21.46	50.18	25.28
4	2h	20.0	45.13	20.23
5	2n	21.21	47.75	22.85
6	3h	19.11	41.24	16.34
7	3n	20.47	44.3	19.4
8	7h	20.98	45.74	20.84
9	Sham-6	18.23	47.3	22.4
10	Orok	16.56	45.9	21
11	Iraq	13.9	36.66	11.76
12	Iba99	13.7	32.52	7.62
13	Iba95	11.76	39.92	15.02
14	Abu Ghraib-3	10.34	35.46	10.56
15	Aadnania	9.45	33.25	8.35
16	Tamoze	10.54	35.8	10.9
17	Alrashid	11.68	38.11	13.21
<i>P</i> value		<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.001

**Table 3.** Matrix of genetic distance between 17 Iraqi wheat genotypes by RAPD markers based on Jaccard coefficient.

Genotypes	Dajla	Furat	1h	2h	2n	3h	3n	7h	Sham-6	Orok	Iraq	Iba99	Iba95	Abu Ghraib-3	Aadnania	Tamoze	Alrashid
Dajla	0																
Furat	0.32384	0															
1h	0.56972	0.28742	0														
2h	0.52774	0.56390	0.42629	0													
2n	0.56573	0.55933	0.66907	0.41748	0												
3h	0.72791	0.58175	0.45033	0.48715	0.49340	0											
3n	0.50461	0.54077	0.49205	0.34082	0.55470	0.56185	0										
7h	0.75204	0.73941	0.67117	0.88891	0.71568	0.60448	0.60125	0									
Sham-6	0.61871	0.60902	0.72639	0.86500	0.53106	0.66304	0.63931	0.35483	0								
Orok	0.72499	0.67018	0.49620	0.60837	0.56298	0.53091	0.51379	0.76111	0.44803	0							
Iraq	0.84059	0.78974	0.73243	0.86146	0.68588	0.50066	0.78511	0.69469	0.64789	0.64497	0						
Iba99	0.80057	0.79228	0.77245	0.99901	0.68508	0.65301	0.82513	0.77245	0.68791	0.64004	0.18312	0					
Iba95	0.72182	0.55798	0.82327	0.82971	0.65413	0.57426	0.75335	0.86773	0.69618	0.68467	0.31399	0.21079	0				
Abu Ghraib-3	0.46348	0.76611	0.78884	0.89911	0.82931	0.63955	0.72374	0.92238	0.74687	0.69280	0.30061	0.21892	0.24780	0			
Aadnania	0.74262	0.40182	0.76229	0.64480	0.67800	0.50583	0.85419	0.80151	0.67775	0.70992	0.35410	0.25220	0.22125	0.26860	0		
Tamoze	0.75171	0.67867	0.33649	0.76528	0.72632	0.48352	0.65564	0.81060	0.89312	0.22470	0.40241	0.30052	0.23182	0.25864	0.20488	0	
Alrashid	0.91697	0.90661	0.84233	0.99141	0.75496	0.50583	0.77723	0.80151	0.71697	0.60814	0.31243	0.21298	0.22125	0.19164	0.24512	0.2219	0



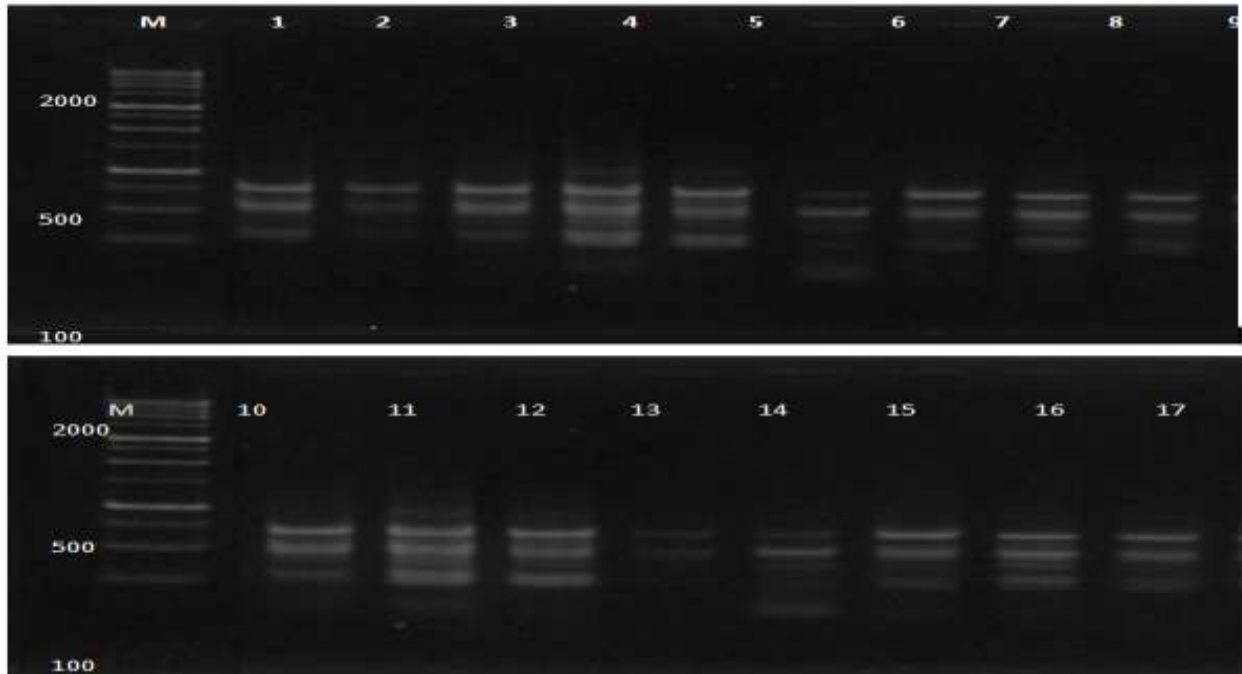
**Figure 1.** Genotypes genetic diversity (phylogenetic tree) resulting from a UPGMA cluster analysis of Iraqi wheat of RAPD marker data. **A** = group 1, **B** = group 2.

**Table 4.** The primers with the number of band products, length of amplified bands, and the number of binding sites.

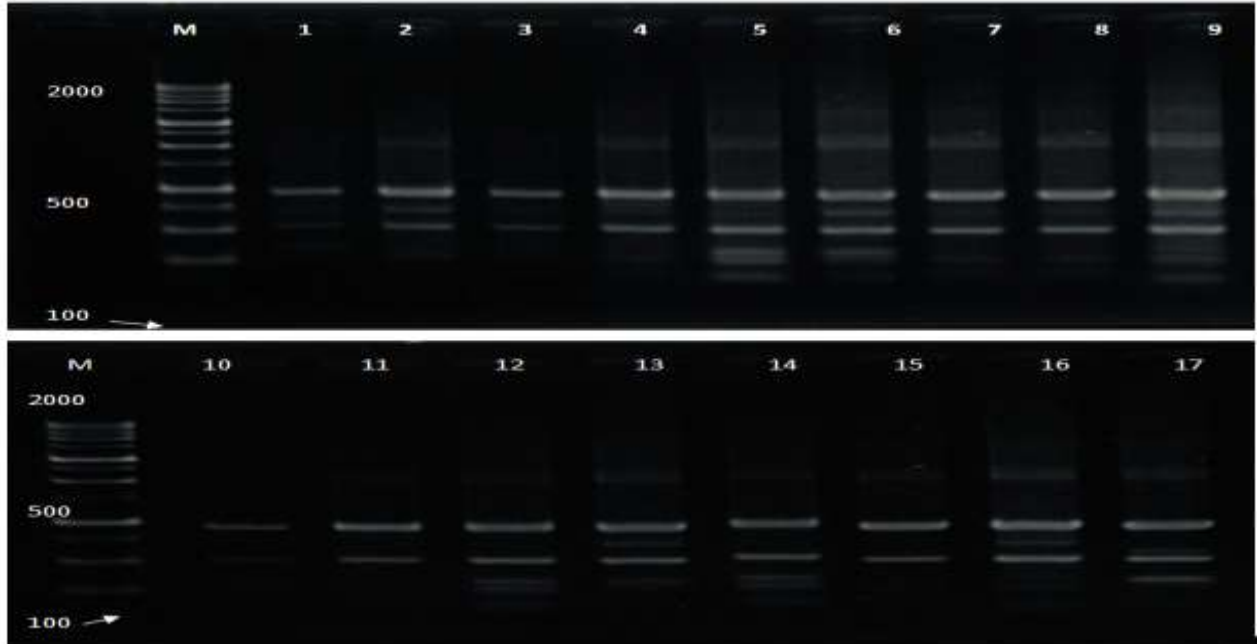
Primers	Length of amplified bands	Number of bands	Number of binding sites
Pr-1	100-300	48	3
Pr-2	100-500	64	6
Pr-3	150-500	55	5
Pr-4	100-500	57	8
Pr-5	100-500	73	8
Pr-6	100-500	68	7
Total		365	37

A summary of the results of amplification profiles RAPD analysis of the 17 wheat genotypes appears in Table 4. The six primers were random and produced several 365 RAPD bands and a total of 37 binding sites with polymorphism bands. The primer Pr-5 gave the largest number of RAPD bands (73), while the Pr-1 primers got the smallest number (48). These results also agree with the past findings on identifying GD and proline content in some Iraqi bread wheat cultivars (Kubba *et*

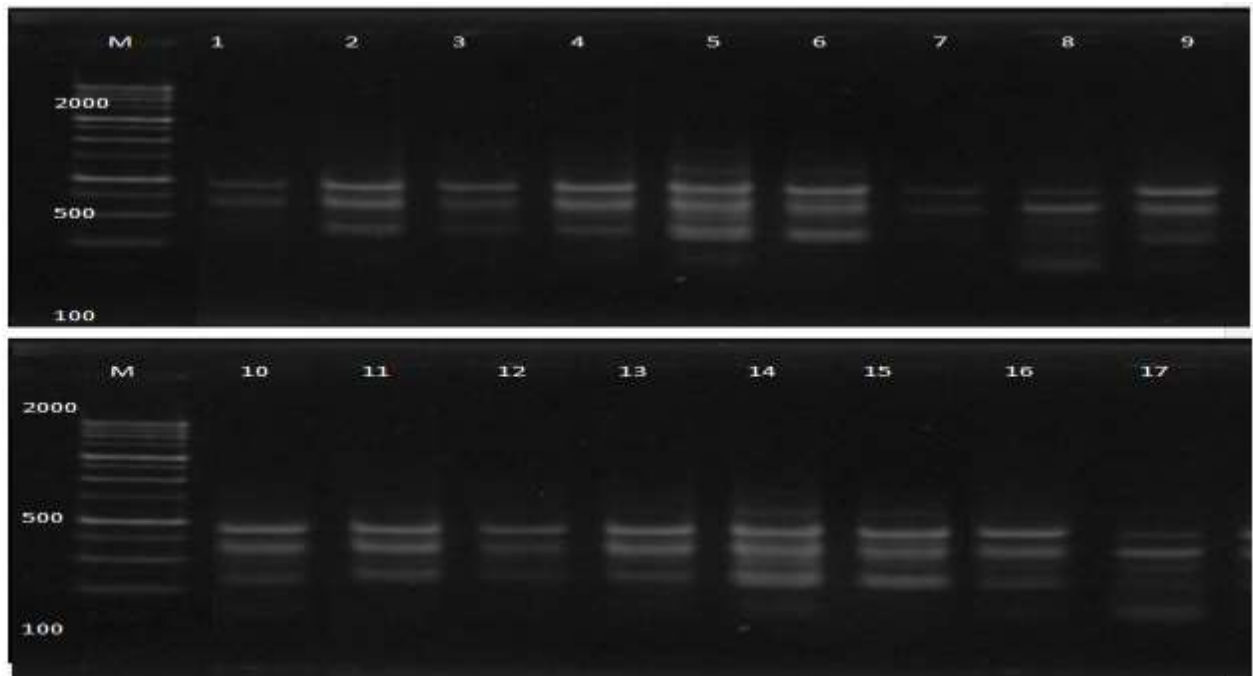
*al.*, 2015; Olgun *et al.*, 2015). The band size was from 100 to 500 bp for the used primers. Banding patterns of the 17 wheat genotypes used the six primers, with the bands of DNA fragments scored as present and absent, as illustrated in Figures 2-7. The aim was to use different primers targeting regions of the gene because it helps to show the difference, if any, between the genotypes according to the initiator sequence used (Zakaria, 2011; Al-Karkhi, 2018).



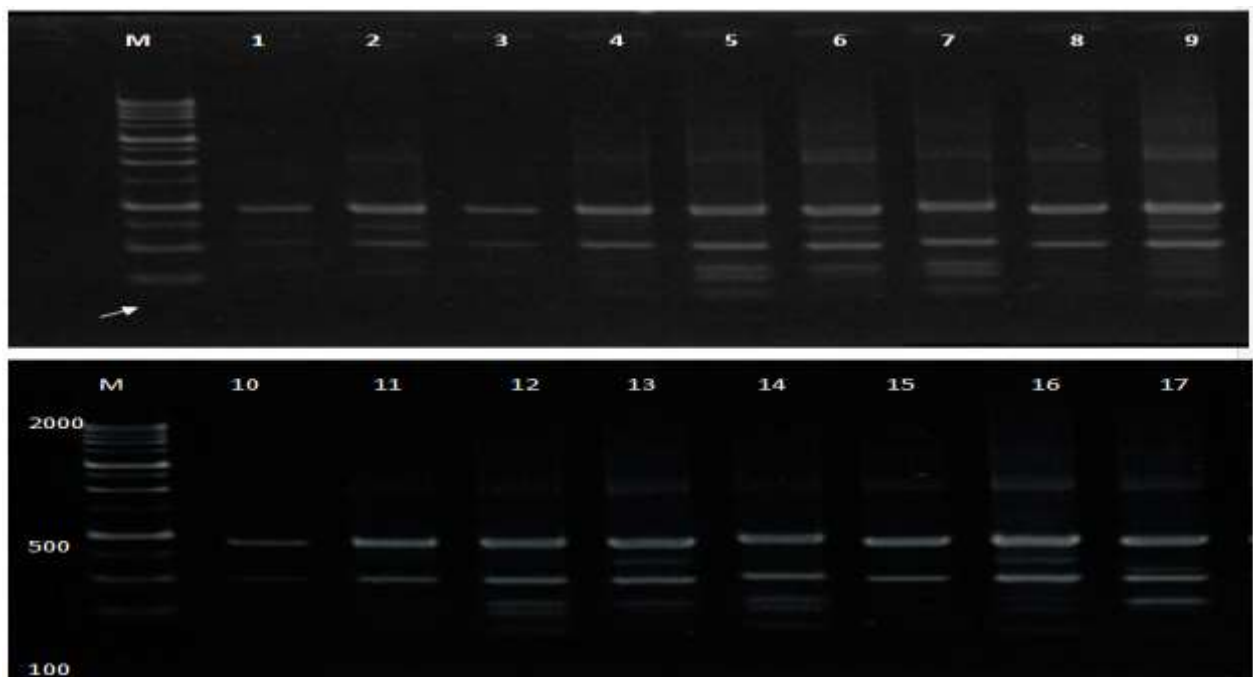
**Figure 2.** Result of gel electrophoresis of PCR products obtained by using (Pr-1) RAPD banding primer, on 1.2% agarose gel, 5V/cm at 1h 30min. For 17 genotypes of wheat, lane M represented the molecular marker M=100bp DNA Ladder Promega.



**Figure 3.** Result of gel electrophoresis of PCR products obtained by using (Pr-2) RAPD banding primer, on 1.2% agarose gel, 5V/cm at 1h 30min. For 17 genotypes of wheat, lane M represented the molecular marker M=100bp DNA Ladder Promega.

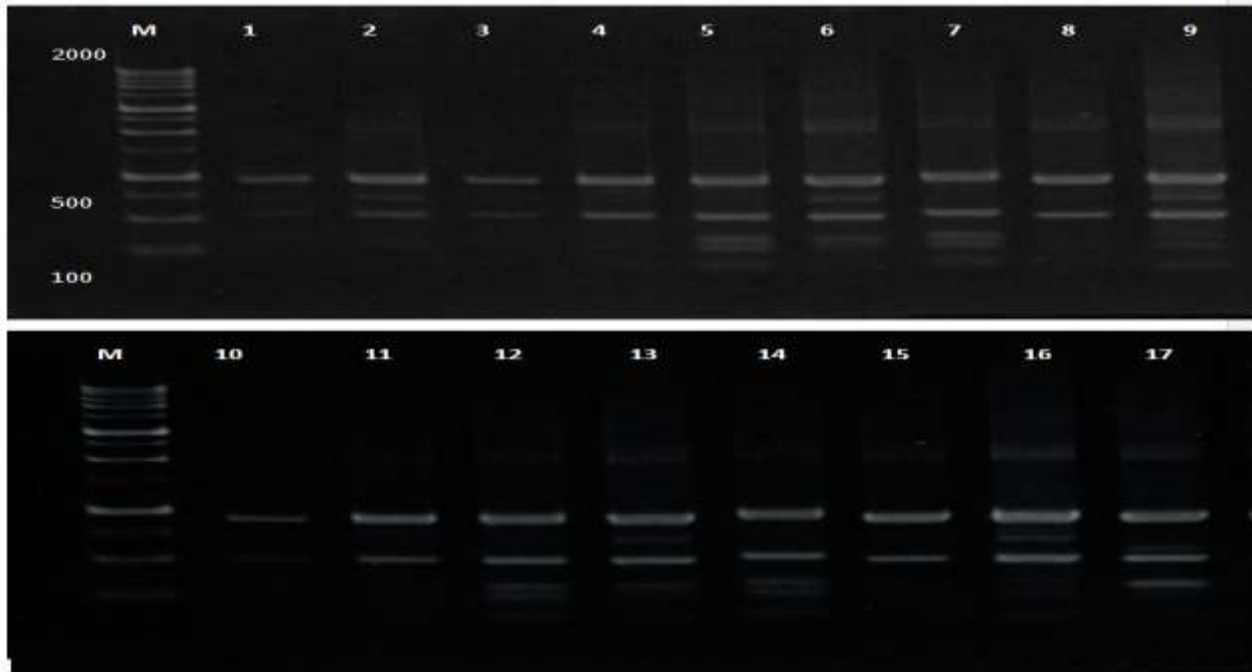


**Figure 4.** Result of gel electrophoresis of PCR products obtained by using (Pr-3) RAPD banding primer, on 1.2% agarose gel, 5V/cm at 1h 30min. For 17 genotypes of wheat, lane M represented the molecular marker M=100bp DNA Ladder Promega.

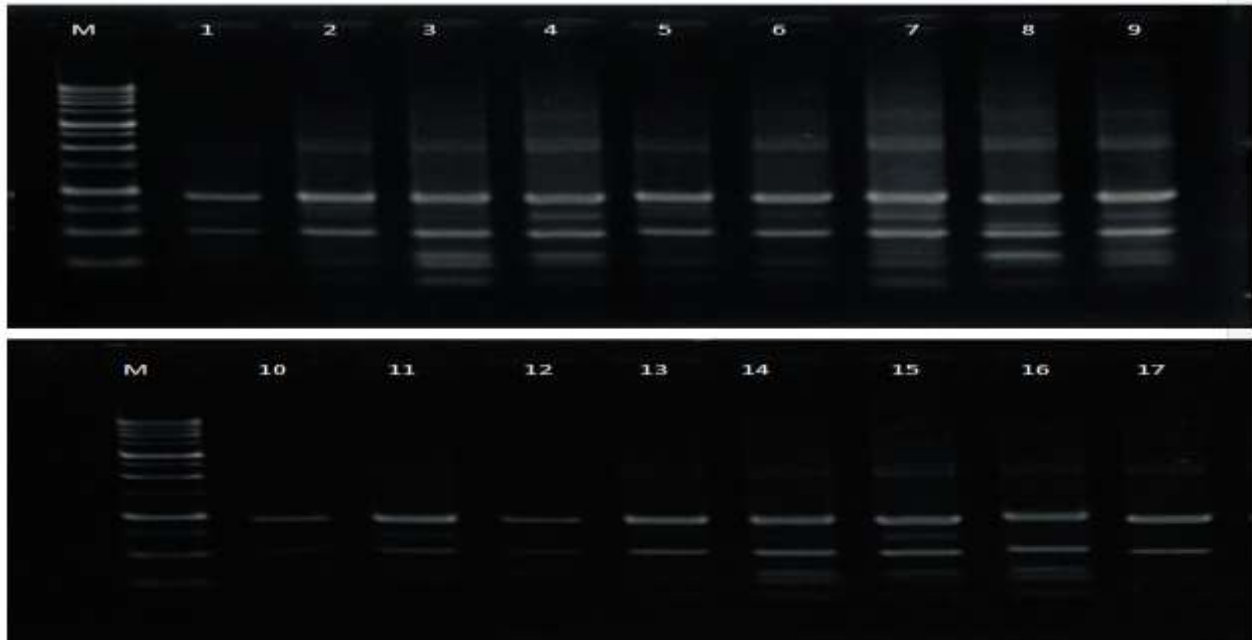


**Figure 5.** Result of gel electrophoresis of PCR products obtained by using (Pr-4) RAPD banding primer, on 1.2% agarose gel, 5V/cm at 1h 30min. For 17 genotypes of wheat, lane M represented the molecular marker M=100bp DNA Ladder Promega.





**Figure 6.** Result of gel electrophoresis of PCR products obtained by using (Pr-5) RAPD banding primer, on 1.2% agarose gel, 5V/cm at 1h 30min. For 17 genotypes of wheat, lane M represented the molecular marker M=100bp DNA Ladder Promega.



**Figure 7.** Result of gel electrophoresis of PCR products obtained by using (Pr-6) RAPD banding primer, on 1.2% agarose gel, 5 V/cm at 1h 30min. For 17 genotypes of wheat, lane M represented the molecular marker M=100bp DNA Ladder Promega.

Cluster analysis means the statistical procedure used in the plant group distribution by their features and then determining the genetic variability degree. The GD dendrogram (phylogenetic tree) built on the RAPD marker exhibited the distribution of selected wheat genotypes in two major groups. The first group included 01 to 10 genotypes (Dajla, Furat, 1h, 2h, 2n, 3h, 3n, 7h, Sham-6, and Orok), while the second group gathered the genotypes 11 to 17 (Iraq, Iba99, Iba95, Abu Ghraib-3, Aadnania, Tamoze, and Alrashid). Furthermore, the first and second groups could further subdivide into two sub-groups. The first group had the subgroup which included the cultivars Dajla, Furat, 1h, 2h, 3n, 2n, and 3h, while the second subgroup included the wheat genotypes 7h, Sham-6, and Orok of the first group. The second group also consists of two sub-groups, with the first subgroup having genotypes Iraq and Iba99, while the second subgroup included genotypes Iba95, Aadnania, Tamoze, Abu Ghraib-3, and Alrashid (Figure 1). The results also authenticated that RAPD markers proved helpful in identifying the wheat populations.

## CONCLUSIONS

The current results confirmed that the wheat genotypes Dajla and Furat provided the highest percentage of protein and wet and dry gluten. Cluster analysis divided all the 17 wheat genotypes into two large clusters, with fragments ranging in size, from 100 bp to 2000 kb.

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