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Research Article

Assessment of Genetic Diversity Among Cabbage (*Brassica oleracea var. capitata*) Genotypes Using RAPD-PCR Markers

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Abstract: Cabbage is one of the important winter vegetables in Iraq, and it belongs to the cruciferous family. The main objective of this study was to investigate the genetic variation of several varieties of cabbage plants. Ten cabbage varieties imported into Iraq as new and exotic genotypes were evaluated. Firstly, the seeds were planted in the fields of Agriculture and Forestry, University of Mosul, and the leaves of ten different types of cabbage were collected. All the samples were processed for DNA isolation and amplified using the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) technique to assess genetic variation within and among the varieties. Five primers obtained from the NCBI database were used for RAPD analysis in the Central Laboratory of the College of Agriculture and Forestry. The RAPD profiles showed variation in the sizes of amplified DNA bands, which ranged from 50 to 3000 bp. In total, 250 bands were recorded, comprising 138 monomorphic bands, 112 polymorphic bands; while there were 5 unique bands. The OPC-08-K primer showed the best variance percentage, primer efficiency, and discriminatory ability, reaching 71.43%, 28.00%, and 36.61%, respectively. The study demonstrates the potential of RAPD-PCR for evaluating genetic diversity and distinguishing different cabbage varieties.

Keywords: *Brassica oleracea var. capitata*, RAPD-PCR, Genetic Diversity, Molecular Markers, Polymorphism.

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1. INTRODUCTION

Domesticated cabbage originated in the Mediterranean region of Europe. Several popular crucifers, such as bok choy, kohlrabi, turnips, Brussels sprouts, cauliflower, mustard, and broccoli, are botanically related to this vegetable [1]. Cabbage was first used by the ancient Greeks and Romans as a treatment for many diseases, including headaches, gout, and mushroom poisoning. Cabbage is still widely regarded as a common market vegetable, despite mounting evidence that cruciferous vegetables can help prevent cancer [2]. In Iraq, cabbage is considered as one of the most important winter vegetables. Per 100 grams of fresh leaves, cabbage contains 94 grams of water, 14 calories, 1 gram of protein, 2 grams of carbohydrates, 260 IU of vitamin A, and 31 milligrams of ascorbic acid [3]. An essential framework for accurate plant taxonomy and identification is provided by integrating morphological and anatomical analysis with DNA fingerprinting techniques. Critical problems about the evolutionary patterns of plant varieties, species delimitation, and intraspecific variation are successfully addressed by this comprehensive approach. The use of high-throughput genotyping in conjunction with thorough phenotypic characterization has recently become a crucial approach for effective extraction and analysis of genetic data [4]. A novel assay for DNA polymorphism is described that amplifies random DNA segments using single primers with arbitrary nucleotide sequences. These polymorphisms can be used for genetic mapping across a variety of species and are identified as amplified DNA fragments that are present in one parent but absent

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in the other and segregate in a Mendelian manner. These polymorphic markers are referred as Random Amplified Polymorphic DNA (RAPD) [5].

Using RAPD analysis, the genetic variability and similarity of head cabbage, cauliflower, Brussels sprouts, broccoli, and kale were successfully assessed [6, 7-12]. Using RAPD, the genetic identity and diversity of fourteen phenotypically homogeneous cabbage accessions (*Brassica oleracea* var. *capitata* L.) referred to as "Golden Acre" were evaluated [13]. Using the RAPD approach, 110 DNA fragments, including 80 polymorphic fragments generated by nine primers, were successfully used to distinguish all 14 accessions. The findings showed that accessions with the same morphological classification differed significantly at the genetic level. RAPD markers provide a useful approach for revealing cryptic genetic variation and providing essential information for the identification, classification, and conservation of cabbage genetic material, particularly for improving the management and application of plant genetic resources [14]. Polymorphic bands, closely related lines, and genetic diversity within and between diploid cabbage lines were all successfully identified by RAPD analysis. These findings confirm that RAPD-PCR is a useful molecular technique for describing the genetic material of cabbage and guiding breeding programs. Further molecular investigation of current genotypes is necessary because the genetic diversity among local cabbage genotypes is still largely unknown [15]. Using RAPD and SSR markers, earlier research examined the genetic diversity and relationships among seven economically significant cabbage cultivars. High polymorphism rates were found in SSR (87.6%) and RAPD (84.44%) markers, indicating substantial genetic variation among the cultivars. Their genetic relationships were successfully clarified, and genotype discrimination was enhanced by analyzing the combined marker data. In this work, RAPD and SSR markers appeared to be useful methods for identifying genetically diverse cabbage cultivars and analyzing genetic variation. The "January King Sel. Improved" and "Golden Acre" cultivars in particular showed significant genetic differentiation, providing important information for further breeding and selection techniques [16]. The novelty of this study is that it is among the first studies to employ standardized RAPD-PCR markers for molecular characterization of recently imported cabbage cultivars in Iraq. It identifies highly informative primers for cultivar discrimination and determines the degree of genetic variability among cultivars. The results offer a useful genetic database for future projects in Iraq aimed at conserving genotypes and supporting cabbage breeding.

2. MATERIALS AND METHODS

2.1. Sample Collection and DNA Extraction

Ten foreign cabbage varieties, presented in Table 1 were included in the present study, which was conducted at the Center of Molecular Genetics Laboratory, College of Agriculture and Forestry, University of Mosul, Iraq, during the fall 2024-2025 growing season. Leaf samples were collected from the vegetable fields of the Horticulture and Landscape Design Department. To prepare mixed samples, leaves were collected from different areas of the green portions of each cabbage variety. Sample collection and DNA extraction were performed according to the instructions provided with the AddBio AddPrep Plant Genomic DNA Extraction Kit.

After adding 20 µl of Proteinase K solution (20 mg/ml) to a 1.5 ml Eppendorf tube, 200 µl of the sample was transferred to the Eppendorf tube containing the enzyme. The sample tube was filled with 200 µl of binding solution, followed by vertexing for 15 seconds and incubation at 56 degrees for 10 minutes. After adding 200 µl of 100% ethanol, the liquid was once again stirred for 15 seconds using the mixer. The lysates were carefully transferred to a spin column and placed in a 2 ml filtrate collection tube. The spin column was centrifuged at 13,000 rpm for one minute and the filtrate was discarded. Then 500 µl of wash 1 solution was added to the drying tube, followed by centrifugation at 13,000 rpm for one minute, and the filtrate was discarded. Similarly, 500 µl of wash 2 solution was added to the drying tube, then centrifuged at 13,000 rpm for one minute, then the filtrate was discarded. The drying tube was centrifuged at 13,000 rpm for one minute to get rid of ethanol residue. The spin column was then transferred to a new Eppendorf tube. Next 75 µl of Elution Solution was added to the drying tube and then centrifuged at 13,000 rpm for one minute.

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Table 1. The sources of cabbage plants.

No.	Name variety	Color	Origin	Source
1	Cavalo cappuccino rose	Red	Italy	From local market, Erbil Kurdistan, Iraq
2	Syrian	Green	Syria	Garden production, Ever Green Company, Syria
3	Copenhagen market	Green	Turkish	Selçuklu/Konya, Turkey
4	Mohrenkopf	Red	Turkish	Selçuklu/Konya, Turkey
5	Yalova sarmalik	Green	Turkish	Selçuklu/Konya, Turkey
6	Kırmızı–tohurau	Red	Turkish	From local market, Erbil, Kurdistan, Iraq
7	Broutis	Green	Franc	State company for agric. suppl., Iraq
8	Brunswick	Green	Italy	U.S.A. subsidiary of Atlantic rich field Asgrow Seed Company
9	Red head	Red	European hybrid cultivar	From local market, Duhok, Kurdistan, Iraq
10	Ruby ball	Red	European hybrid cultivar	From local market, Duhok, Kurdistan, Iraq

All ten variety samples were stored in aluminum foil at 4 °C until DNA extraction. The extracted DNA was stored at -20 °C for downstream applications. The quantity and quality of DNA were assessed using gel electrophoresis. Finally, the samples of the eluted DNA extract were stored frozen in Eppendorf tube until use.

2.2. PCR Primer

Five standard arbitrary decamer primers from the Operon Technologies RAPD primer sets were used for RAPD-PCR analysis. These primers had been previously reported and successfully used in genetic diversity studies of *Brassica* species. The primers were synthesized or obtained commercially and used according to established RAPD-PCR protocols to characterize the genetic variation among the cabbage (*Brassica oleracea* L.) [5, 17]. Table 2 displays the primer sequences that were employed.

2.3. PCR Amplification

Five random primers were used for RAPD-PCR amplification using (Applied Biosystems® Veriti® 32-Well Fast Thermal Cycler, USA). The total reaction volume for each reaction sample was 50µl. The conditions of the PCR program were run, firstly an initial denaturation at 98 °C for 60 seconds, followed by 35 cycles of denaturation at 96 °C for 30 seconds, annealing between 34 to 36 °C for 30 seconds, and then extension at 72 °C for 60 seconds, with final extension at 72 °C for 5 min. The amplified products were separated on a 1% agarose gel, stained with safe stain and visualized under UV transillumination using a Biostep-UST-20M-8K imaging system. All amplification reactions were independently repeated more than once under identical experimental conditions. Only clear, consistent, and reproducible banding patterns observed across the repeated experiments were included in the final analysis (Table 3).

Table 2. Sequence of five random primers used to detect genetic variation of cabbage plants.

'5-3' sequence	Primers name
'5-TGGACCGGTG-3'	OPC-08-K
'5-GTGATCGCAG-3'	OPA-10
'5-CTGCTGGGAC-3'	OPB-10
'5-AGATGCAGCC-3'	OPE-06
'5-ACGGCGTATG-3'	OPE-19

2.4. Data Analysis Details

A binary data matrix was created by assessing the amplified RAPD bands according to their presence

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Table 3. RAPD-PCR reaction mixture for amplification of the five primers for ten imported cultivars.

Reaction phase	Temperature (°C)	Time (s)	Number of circles	Reaction components	Added quantities (µl)
Initial denaturation	98	60	1	Primers	1
Denaturation	96	30	35	DNA	3
Annealing	34-36	30		Master Mix	20
Extension	72	60		Free Water	26
End	72	300			

(1) or absence (0) across the cabbage genotypes. Genetic similarity and genetic distance between the genotypes under study were calculated using the binary matrix. MEGA11 software was then used to perform a cluster analysis on the generated genetic distance matrix. To identify the genetic links and clustering patterns among the cabbage genotypes, a dendrogram was built using the obtained genetic distances.

3. RESULTS

Figure 1 shows the genomic DNA of the cabbage varieties separated on a 1% agarose gel (1g of agarose in 100 ml of TBE buffer 1X) during electrophoresis to evaluate the quality of the isolated DNA obtained using the Add Prep Plant Genomic DNA Extraction Kit. The electrophoresis device works to separate the bands according to the molecular sizes (base pairs). Smaller DNA fragments migrate farther and faster through the gel than larger DNA fragments. As shown in Figure 1, the electrophoresis results indicate that the DNA bands varied in their positions and intensities because they are of good quality and have different partial sizes. The quality of the DNA was estimated through the fluorescence of the samples after staining with safe stain, which shows the genomic DNA bands isolated from the cabbage under study.

3.1. DNA Random amplified polymorphic

The RAPD technique was used in the study to identify the genetic differences among the cabbage varieties under investigation and to determine the genetic distance between them, as well as to construct a phylogenetic tree, based on the presence or absence of amplified bands and variations in the

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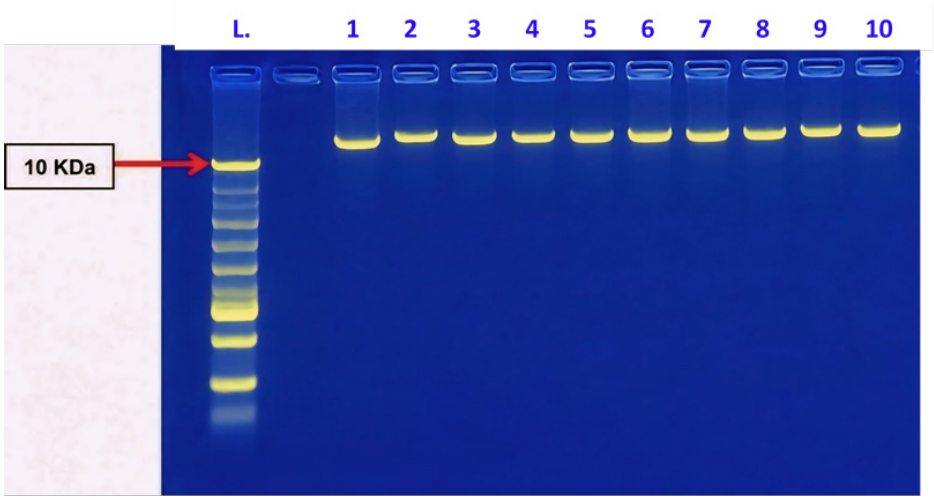


Fig. 1. The genomic DNA in the Cabbage varieties. 10 ul DNA samples isolated from the cabbage varieties on 1%agarose gel, namely (L= DNA ladder marker (10000 bp), 1 = Cavalo cappuccino rose, 2 = Syrian, 3 = Copenhagen market, 4 = Mohrenkopf, 5 = Yalovasarmalik, 6 = Kirmizi-tohurau, 7 = routis, 8 = Brunswick, 9 = Red head, and 10 = Ruby).

molecular sizes of these bands. The relationship among the genetic differences or variation between these varieties was then determined. Because RAPD results can be influenced by variations in reaction components and experimental conditions, careful optimization is necessary. Factors such as temperature, primer concentration, genomic DNA concentration, Mg^{2+} ion concentration, and pipetting accuracy should be carefully controlled to ensure consistent and reproducible results.

Five random primers were employed in this investigation because different cabbage varieties showed variations in the number and locations of polymorphic bands, which allowed us to identify genetic variation. The total number of bands generated by these primers were also determined, as certain primers produced both absent and distinct bands, which ranged from 50-3000 bp. The total number of loci identified by these primers across the genomes of the samples was 25, including 12 monomorphic sites and 13 polymorphic sites, and the total bands are 250, of which 138 were monomorphic bands and 112 polymorphic bands; the results for these primers are presented in Table 4.

3.2. OPC-08-K

This primer produced 41 polymorphic bands that differed in their location and molecular size, as their molecular sizes ranged between 50-2000 bp, while the number of bands for each variety ranged between 1-4; varieties 3, 7, and 9 showed the least number of bands, and varieties 1, 2, 4, 5, and 6 showed 3 bands, while varieties 8 and 10 showed the highest number of bands, with four bands each. Variety 8 showed a unique band at 1000 bp, variety 1 showed a distinct band at 750 bp, and variety 10 showed unique band at 250 bp. These unique bands may serve as diagnostic markers for distinguishing these varieties from the others. In addition, this primer showed an absent band in variety 1 at a size of 50 bp, shown in Figure 2 and Table 4. The contrast ratio of this primer was 71.43%, and the relative efficiency was 28%. The discriminatory ability of this primer was 36.61%, as shown in Table 4.

3.3. OPA-10

The results of the PCR amplification using this primer showed 18 polymorphic bands that varied in position and molecular size, with molecular sizes ranging between 50-500 bp. The number of fragments for each variety ranged from 2-5. Varieties 1, 4, and 5 were characterized by the least number of bands. Cultivars 2, 7, 8, and 10 showed 4 bands each, while cultivar 9 showed the highest number, with 5 bands. This primer showed 3 polymorphic sites, and no unique bands appeared for this primer, as shown in Figure 3 and Table 4. The percentage of variation was 60%, while its efficiency was 20%. The discriminatory ability of the primer was 16.07%, as shown in Table 4.

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Table 4. The results of prefixes used in RAPD-PCR reactions.

Primer Sequence	Name of Primer	Molecular Weight bp	The number of sites produced	Number of sites Polymorphic bands	Total band	Monomorphic band	Polymorphic bands	Unique band	Variance percentage %	Primer efficiency %	Discriminatory ability %
1	OPC-08-K	3000-50	7	5	70	29	41	3	71.43	28.00	36.61
2	OPA-10	500-50	5	3	50	32	18	0	60.00	20.00	16.07
3	OPB-10	600-100	4	1	40	30	10	0	25.00	16.00	8.93
4	OPE-06	500-50	4	1	40	26	14	0	25.00	16.00	12.50
5	OPE-19	1500-100	5	3	50	21	29	2	60.00	20.00	25.89
Total			25	13	250	138	112	5			

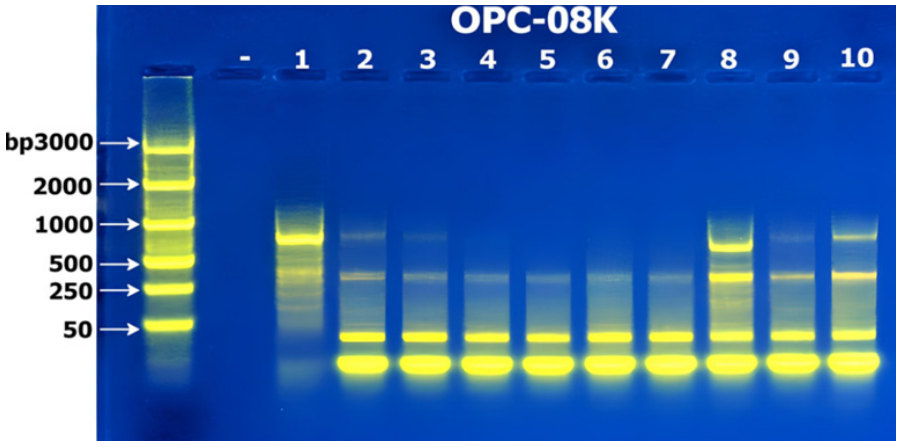


Fig. 2. Primer OPC-OB K.

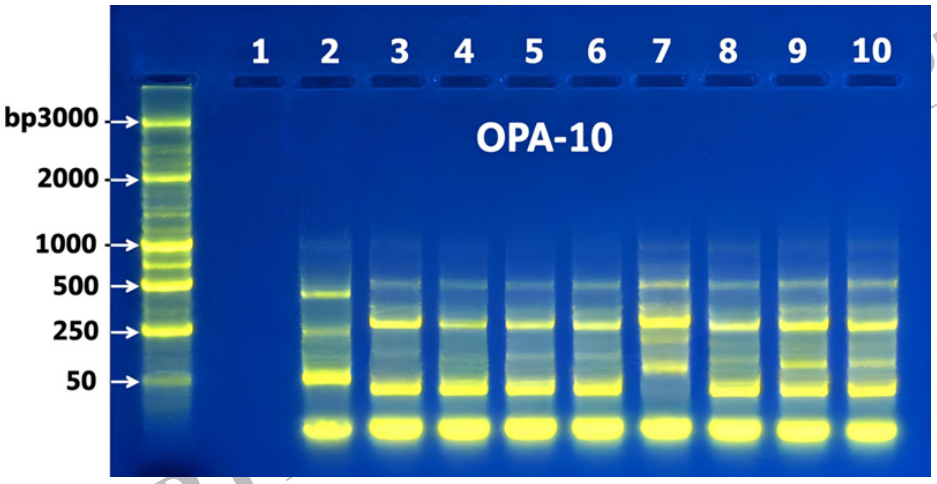


Fig. 3. Primer OPA-10.

3.4. OPB-10 Primer

Agarose gel electrophoresis revealed a total of 40 polymorphic bands ranging in molecular size from 100-600 bp, including 30 monomorphic and 10 polymorphic bands. The bands observed in varieties 1 and 5 ranged from 100-250 bp, whereas those in variety 4 ranged from 50-100 bp. The smaller DNA fragments migrated farther through the agarose gel. The highest number of bands observed in variety 9, with 5 bands ranging from 50-500 bp and varieties 7, 8, and 10 showed 4 bands with different sizes and weights. These results indicate that the primer produced different banding patterns among the cabbage varieties, reflecting genetic variation among them that may contribute to differences in phenotypic and head-related productive characteristics, as shown in Figure 4 and Table 4. No unique bands were detected for this primer. The percentage of variation was 25%, the efficiency was 16%, and the discriminatory ability was 8.93%.

3.5. OPE-06 Primer

Through the results of electrophoresis, this primer showed 14 different polymorphic bands in their locations and molecular sizes, as their sizes ranged between 50-500 bp. Three bands with sizes ranging from 100-250 bp were observed in varieties 1, 2, 3, 4, 7, 8, 9, and 10, while no band appeared in variety 6. These are the markers that this sequence has the same location on the genomic DNA that the studied varieties of cabbage did not distinguish this primer from any unique band among the varieties (Table 4 and Figure 5). The percentage of variation for this primer reached 25% and its efficiency was 16% while its discriminatory ability was 12%.

3.6. OPE-19 Primer

The results of the PCR interaction of this primer with the genomic DNA showed 29 distinct polymorphic bands in their locations and sizes ranging from to

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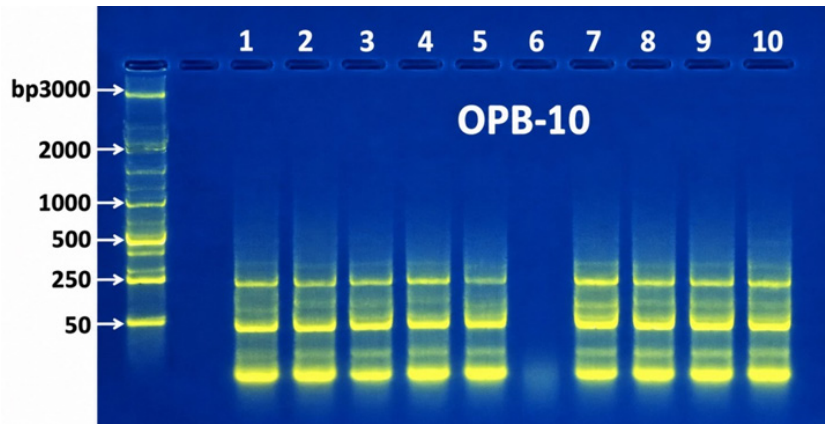


Fig. 4. Primer OPB-10.

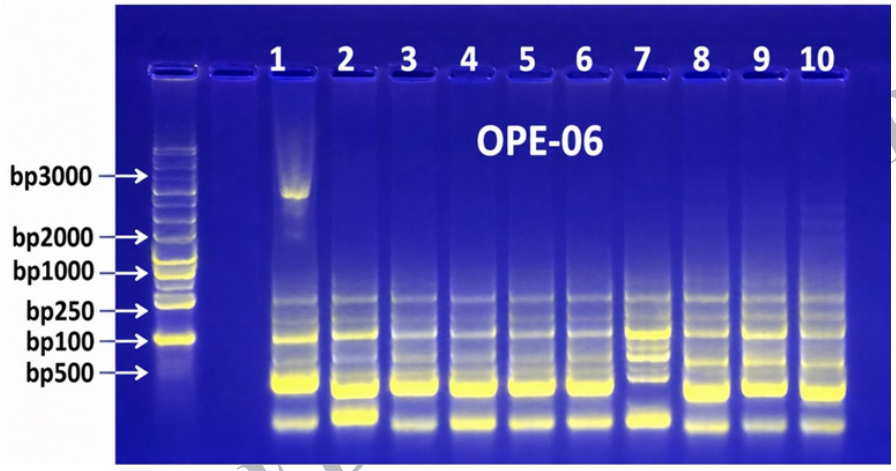


Fig. 5. Primer OPE-06.

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100-1500 bp, and the number of bands for each variety were between 1-4 bands. However, no band appeared at lane 6, meaning that the sequence did not obtain any corresponding band for it in this lane. For this marker, the sequence has binding sites of different sizes and molecular weights of DNA, and this sequence showed only one unique band, at a size of 1500 bp and another at 300bp as shown in Table 4 and Figure 6.

The percentage of contrast was 60%, and its efficiency was 20%, while its discriminatory ability was 25.89%. The genetic distance, presented in Table 5, was also estimated based on the RAPD markers among the cabbage cultivars under study, by using the NTSYS genetic analysis program [18]. In accordance with established RAPD-based methods previously used for *Brassica oleracea* genotypes, including cabbage (*B. oleracea* var. *capitata*), genetic similarity and genetic distance among the cabbage genotypes were assessed using the binary RAPD data matrix. The previous studies used 62 RAPD primers in length 10mer, and used the RAPD data to estimate Nei–Li genetic similarity between pairs of genotypes, genetic distances, and constructed dendrograms to compare genetic relationships. The study found a correlation between the genetic similarity estimates obtained from RAPD, with a rank correlation coefficient of 0.745, and concluded that RAPD provides a suitable level of discrimination for determining genetic relationships between *B. oleracea* genotypes. [19].

The genetic distance ranged between 0.061 and 0.746. In the case of genetic similarity between any two cultivars under study, a genetic distance of zero indicates that the cultivars are genetically identical, with the similarity rate of 100%. The least genetic distance was seen among cultivars 1 and 3, reaching up to 0.061. This is an indicator that these two cultivars have the largest percentage of closeness or similarity in genetic traits, depending on the primers that were used in this study. It also appears from Table 5 that the highest genetic distances were

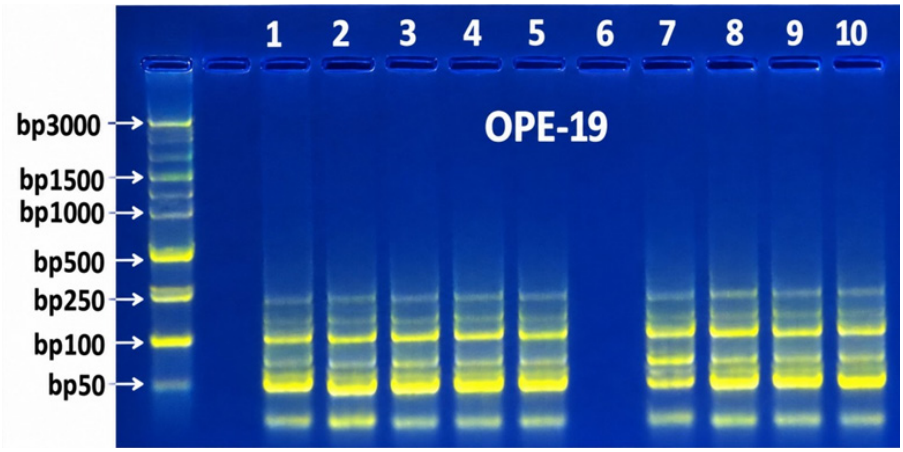


Fig. 6. Primer OPE-19.

Table 5. Genetic distant between the ten genotypes of cabbage.

Genotypes*	1	2	3	4	5	6	7	8	9	10
1	0.000									
2	0.214	0.000								
3	0.061	0.213	0.000							
4	0.251	0.117	0.251	0.000						
5	0.371	0.354	0.371	0.391	0.000					
6	0.356	0.328	0.356	0.259	0.314	0.000				
7	0.251	0.405	0.251	0.442	0.391	0.259	0.000			
8	0.488	0.237	0.485	0.391	0.511	0.311	0.274	0.000		
9	0.286	0.334	0.280	0.371	0.308	0.199	0.189	0.308	0.000	
10	0.405	0.589	0.400	0.509	0.746	0.432	0.403	0.746	0.255	0.000

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observed among cultivars 10 and 5, 10 and 8, and 5 and 8, which ranged up to 0.746, 0.746, and 0.511, respectively. Between grade 10 and cultivars 2 and 4, the genetic differences were 0.589 and 0.509, respectively; the lowest convergence ratio appeared between cultivars 8 and 5, which amounted to 0.511. Figure 7 shows the cluster dendrogram analysis, created using MEGA11 software based on genetic distance analysis between the cultivars under study to determine the genetic relationship among these cultivars in the form of clusters.

The results of DNA marker interactions between the genetic variants of cabbage under study, together with the genetic distance matrix shown in Figure 7, were used to assess the genetic distances between the cultivars. The genetic distance values varied from a minimum of 0.061 to a maximum of 0.746. There is a limit to the extent of the genetic distance values because the study's genetically included varieties that share the fewest bands due to variations in the nucleotide sequence in those varieties' genomes. This emphasizes how crucial it is to use diverse primers that target different parts of the genome, which will cause disparities. The

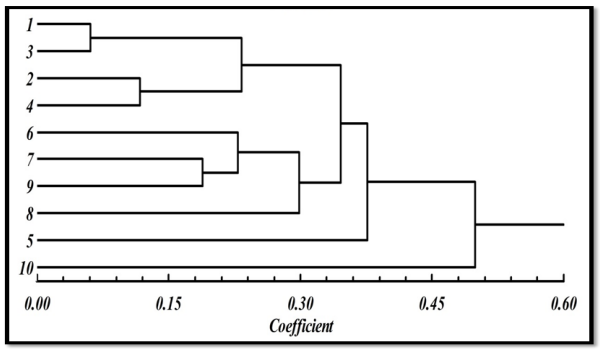


Fig. 7. Cluster analysis Dendogram between the ten genotypes of cabbage using MEGA11 software analysis.

reason is the initiator's sequence and the extent to which the genomes of the semi-cabbage under investigation differ from those of other cabbage varieties. The current study's genetic distance findings are consistent with earlier RAPD-based evaluations of cabbage genetic diversity. RAPD markers were used to report genetic distances of 0.4057 and 0.3273 among local cabbage (*B. oleracea* var. *capitata*) varieties. The genetic distance in the current study varied from 0.061 to 0.746, indicating a greater range of genetic relatedness among the evaluated cabbage genotypes [20].

The present findings are comparable to those reported by Al-Tameme [21], who used RAPD markers to identify significant genetic variation among *Brassicaceae* taxa. The previous study found 26% polymorphism among the studied taxa; however, the present study revealed a different level of polymorphism, reflecting changes in the genetic material and degree of relatedness among the cabbage genotypes examined. The arrangement of varieties, depending on the genetic distance values (Table 5), and the genetic dendrogram tree (Figure 7), or the dendrogram cluster analysis, depends on the values of the genetic range to which the main groups are related [22-24].

4. DISCUSSION

Through the results of the primers used in this study, which are shown in Table 2. The PCR operating conditions are presented in Table 3. A temperature of 34-36 °C was chosen for the second cycle at annealing step to suit the primers used, which contain a small number of nucleotides and therefore do not require a high annealing temperature, making them very suitable for the RAPD-PCR technique used in this study. Different types and patterns of fragments (bands) were observed. The total number of loci recognized by the five primers in the genome of the samples was 25, with an average of 5 sites per primer. Of these, 12 were monomorphic sites, with an average of 2.4 loci for each marker while 13 were divergent sites, with an average of 2.6 loci per primer. The primers OPB-10 and OPE-06 were characterized by the lowest number of polymorphic sites, reaching 1 site, while the highest number of sites were observed with the primer OPC-08-K. This result was consistent with the previous findings [24]. The initiator OPC-08-K produced the highest number of identified loci, consistent with previous studies [25, 26]. The total number of bands produced by these sites, are shown in Table 4, amounted to 250, including 138 monomorphic bands and 112 polymorphic bands. The primer OPA-10 produced the highest number of monomorphic bands, amounting to 32, and the lowest number of bands was obtained with the initiator, OPE-19, amounting to 21 monomorphic bands. The number of bands displayed by each cabbage variety provides information about the number of loci detected and amplified by the RAPD markers. These variations in the number, types, and sizes of bundles in the varieties of the type of cabbage under study may be caused by the primer's ability to identify and determine the genetic difference among that affect regions between or within primer-binding sites and naturally occur during the evolutionary process of living organisms. These variations can also result from deletions, substitutions, or additions at the sites to which the primer binds [26].

Additionally, some of the cabbage varieties under study were identified by the presence or absence of distinctive bands, which are displayed in Table 4. For the primers employed in this investigation, the sum of the resulting distinctive bands was 8 distinct bands, comprising 3 absent bands and 5 unique bands. Cultivar 8 was identified by a distinct band at the starter OP-80-K and at a molecular size of 1000 bp, and cultivar 9 was identified at the same beginning by a distinct band at a molecular size of 250 bp (DNA stander). These bands are among the most significant diagnostic indicators that rely on polymorphism among the varieties of cabbage. For cultivar 1 at 1500 bp and cultivar 7 at 300 bp, the primer OPE-19 displayed a distinct band.

The presence and observation of these bands in the cultivars are among the most significant characteristics that distinguish them from one another. The appearance of these bands in one cultivar but not the other indicates the presence of the corresponding DNA sequence in the genomic DNA of that cultivar rather than other cultivars. This may indicate the presence of mutations at a particular location that resulted in the primer's binding and the formation of the characteristic bands [23-26]. The absence of bands and their occurrence in a specific variety without other variations serve as a useful indicator and guide for this class's differentiation. In variety 1 at a molecular size of bp 50 and variety

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3 at a molecular weight of bp 500, the primer OPC-08-K displayed a missing band. When the OPE-06 primer is used, all packages show up in Class 6, and when the OPE-19 primer is used, they show up in Classes 3 and 9.

RAPD markers were used to access the genetic identities and relationships of cabbage (*Brassica oleracea*). Among the cabbage genotypes, 117 of the 143 polymorphic fragments generated by 25 decamer primers were identified. Based on Nei and Li [18] coefficient, the previous studies estimated genetic similarity using RAPD banding patterns. Their findings showed the efficacy of RAPD markers for identifying specific genotypes and determining the genetic relationships between cabbage lines. Similarly, RAPD analysis showed significant genetic variation among the cabbage genotypes under investigation, with genetic distance values ranging from 0.061 to 0.746. This substantial variation supports the utility of RAPD markers for detecting genetic differentiation and analyzing genetic relationships within cabbage genotypes [27]. The current research in consistent with the findings of Lázaro and Aguinagalde [17], who used RAPD markers to show significant molecular variation among *Brassica oleracea* populations. RAPD analysis of the cabbage genotypes under investigation in this work also showed significant polymorphism, demonstrating the usefulness of RAPD markers in identifying molecular variations within *B. oleracea*. An additional method of conventional morphological evaluation of genetic diversity in cabbage and other *Brassica* crops is molecular characterization. The genetic diversity and identification of *B. oleracea* cultivars have also been effectively assessed using molecular markers such as SSR [28]. The existence or absence of bands is a characteristic that sets cultivars apart, as Table 4 illustrates; it might have taxonomic significance. This could be because the cultivar's DNA sequence lacks appropriate binding sites for the primer, which can be used to create cultivar differences [29].

The efficiency of the primers differed in their ability to reveal differences among the varieties under study. The highest efficiency was observed for primer OPC-08-K, which amounted to 28%, while the lowest efficiencies were observed for primers OPB-10 and OPE-06 at 16% for both. The discriminative ability of the primer OPC-08-K was the highest at 36.615 and the lowest discriminatory ability was observed for primer OPB-10, which amounted to 8.93%. These results indicate that primer efficiency increases with the increase in the number of bands generated by PCR-RAPD amplification [30-32]. The results of this study offer new molecular evidence for evaluating genetic diversity among the genotypes of cabbage that were assessed by using these primers as specific primers OPC-08-K. The discovered polymorphic RAPD markers can be useful molecular tools for characterizing genetic material, selecting parents in breeding programs, and developing improved cultivars with desired agronomic characteristics. Additionally, the genetic diversity found in this study supports future breeding programs and biodiversity preservation by aiding in the protection and sustainable management of cabbage genetic resources.

5. CONCLUSIONS

The study demonstrated that genetic distance values ranged from 0.061 to 0.746, and molecular marker analysis effectively distinguished among the investigated cabbage cultivars and demonstrated a significant level of genetic variation. The identified variation highlights the usefulness of DNA-based markers for determining genetic links and reflects underlying genomic differences among cultivars. The analysis's reliability is further confirmed by the correlation between the Dendogram clustering and the genetic distance matrix. Additionally, the Dendrogram's clustering pattern matched the genetic distance matrix, demonstrating the accuracy of the molecular data in distinguishing cabbage cultivars and clarifying their genetic links. These findings offer a solid basis for the conservation of genetic resources, the identification of genetically unique parental lines, and the creation of breeding plans targeted at increasing genetic improvement in cabbage.

6. ACKNOWLEDGMENTS

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7. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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8. FUNDING

No funding was received to conduct this research.

9. ETHICAL STATEMENT

This study does not involve human participants or animal subjects. Therefore, ethical approval was not required.

10. DECLARATION

The authors declare that the results presented in this manuscript are original and that the same material has neither been published previously nor is currently under consideration for publication elsewhere. All authors have approved the final version of the manuscript and agree to its submission for publication. In case the article is accepted for publication, its copyright will be assigned to the Pakistan Academy of Sciences. The authors confirm that, where necessary, permission has been obtained to reproduce copyrighted material from other sources and that no copyrights have been infringed.

11. AUTHORSHIP CONTRIBUTION

A.A. came up with the research hypothesis, produced the manuscript, and analyzed the data; H.A.S. assisted with experimentation and conceptualization; and A.M.A.S. assisted with editing and data analysis.

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